Application Notebook

GC and GC-MS Applications for Food Safety Analysis

- Pesticides
- Additional Contaminants
- Natural Compounds and Food Additives
- About GC and GC-MS Technology

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GC and GC-MS Applications for Food Safety Analysis

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Application Notebook

Pesticides

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Extraction of Organochlorine Pesticides from Oyster Tissue Using Accelerated Solvent Extraction

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Key Words
Persistent organic pollutants, moisture absorbing polymer, wet samples, accelerated solvent extraction, sample preparation

Introduction

Organochlorine pesticides (OCPs) are a class of chemicals that were used to control insect pests since the 1940s. The use of OCPs was banned in the later part of the last century due to their longevity, a trait that made them effective for long term pest control, but also increased concerns of potential health outcomes such as cancer in humans and ecosystem disruption. Pesticides are regulated in the U.S. by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Some states also regulate pesticides under FIFRA, in a more restrictive manner than the EPA. In the European Union, water intended for human consumption must meet a maximum level of 0.1 µg/L for each pesticide and a maximum of 0.5 µg/L for total pesticides, except for aldrin, dieldrin, heptachlor, and heptachlor epoxide, which are each limited to maximum levels of 0.03 µg/L. Maximum contaminant levels have been established for OCPs by the United States Environmental Protection Agency ranging from 0.2 µg/L for Lindane to 2 µg/L for Endrin.

Many OCPs are endocrine disrupting chemicals, meaning they have subtle toxic effects on the body's hormonal systems. Endocrine disrupting chemicals often mimic the body's natural hormones, disrupting normal functions contributing to adverse health effects. OCPs are persistent organic pollutants (POPs), a class of chemicals that are ubiquitous environmental contaminants because they break down very slowly in the environment and accumulate in lipid rich tissue such as body fat. According to the Centers for Disease Control and Prevention (CDC), most people have OCPs present in their bodies. Exposure to low concentrations of organochlorine chemicals over a long period may eventually lead to a substantial body burden of toxic chemicals. Organochlorine compounds have long been recognized as the most deleterious contaminants to biota in the world's marine and estuarine waters. Various biomonitoring strategies have therefore been developed to monitor and evaluate the adverse impact of these compounds on the marine ecosystems. Analyses of OCPs are becoming increasingly important,

and often with the need to isolate and analyze trace levels of compounds from a variety of matrices such as soil, sediment, animal tissue, fruits, and vegetables. Sample pretreatment constitutes an important step prior to analysis. The purpose of the sample pretreatment step is to selectively isolate the analytes of interest from matrix components and present a sample suited for routine analysis by an established analytical techniques such as gas chromatography or high-pressure liquid chromatography. Accelerated solvent extraction is an established technique for extracting analytes of interest from a solid, semisolid or an adsorbed liquid sample using an organic solvent at an elevated temperature and pressure. The elevated pressure elevates the boiling temperature of the solvent thereby allowing faster extractions to be conducted at relatively high temperatures. Thus the extraction process is significantly faster than traditional methods such as Soxhlet extraction.

This Application Brief discusses the use of Thermo Scientific™ Dionex™ ASE Prep MAP, a proprietary polymer designed to remove moisture and increase extraction efficiencies from wet samples including soils, tissues and food products. This polymer is useful for in-cell extraction of trace level organics from a variety of moisture containing samples with no additional pre or post extraction steps. The Dionex ASE Prep MAP polymer has a high-capacity for water removal and does not suffer from some of the limitations of clumping or precipitation observed in some of the traditional drying methods.

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Equipment

- Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system, equipped with 34 mL Stainless Steel Extraction Cell Kit, (P/N 060071)
- Filters, Glass Fiber Cell (P/N 056781)
- 250 mL Clear Collection Bottles (P/N 056284)
- Analytical Balance (read to the nearest 0.001 g or better)
- Mortar and Pestle (Fisher Scientific or equivalent)
- Gas Chromatograph (GC) with Electron-Capture Detector (ECD)

Consumables, Regents and Standards

- Dionex ASE Prep Map, Moisture Absorbing Polymer (P/N 083475)
- Thermo Scientific Dionex ASE Prep DE (diatomaceous earth) Dispersant, 1 kg Bottle (P/N 062819)
- Sodium Sulfate
- Acetone
- Hexane
- Heptachlor
- Lindane
- Aldrin
- Dieldrin
- Endrin
- Dichlorodiphenyltrichloroethane (DDT)

All solvents are optima-grade or equivalent and are available at Fisher Scientific.

Sample Preparation and Experimental Conditions

Sample Preparation Using Sodium Sulfate as the Drying Agent

The Oyster samples were prepared by blending or chopping to produce a unifrom homogenate. 2.5 g of the spiked oyster sample was treated with 9 g of sodium sulfate as the drying agent prior to in-cell extraction in the Dionex ASE 350 system. The extraction was pursued at 100 °C using hexane:acetone (1:1) as solvents. The extracts were analyzed by GC-ECD.

Sample Preparation Using Dionex ASE Prep MAP as the Drying Agent

A 5 g portion of the homogenate was accurately weighed and mixed with 1.7 g of Dionex ASE Prep DE and 1.7 g of Dionex ASE Prep MAP. Carefully transfer the samples to the extraction cells, ensuring that the sample is completely removed from the container. Load the extraction cells and collection vials into the Dionex ASE 350 system and perform the extraction according to the conditions listed. In the case of spiked samples the spikes were added to the sample prior to extraction.

Accelerated Solvent Extraction Conditions	
Oven Temperature:	100 °C
Pressure:	1500 psi
Static Time:	5 min
Static Cycles:	3
Rinse Volume:	60%
Solvent:	Hexane/Acetone (1:1, v/v)
Total Extraction Time:	22-25 min

Results and Discussion

Sample preparation is challenging for a wet animal tissue sample such as an oyster sample. The presence of water in such a sample can result in poor recoveries of the analyte of interest. A drying step is therefore needed before the extraction. Mixtures of six OCPs at concentrations of 500 ng/g each were spiked on to the wet oyster samples. The spiked oyster samples were mixed with Dionex ASE Prep MAP and Dionex ASE Prep DE (1:1) or mixed with sodium sulfate as the drying agent prior to in-cell extraction in the Dionex ASE system. The extraction was pursued at 100 °C using hexane: acetone (1:1) as solvents. The extracts were analyzed by GC-ECD. The results in Table 1 show recoveries ranging from 91% for Lindane to 114% for DDT when the extractions are done using Dionex ASE Prep MAP and Dionex ASE Prep DE. The recoveries for extractions done with sodium sulfate are considerably lower ranging from 69% for DDT to 81% for Lindane. The data shows that Dionex ASE Prep DE and Dionex ASE Prep MAP were an effective drying agent for wet oyster samples with excellent recoveries for the six OCPs. In contrast the sodium sulfate treated sample showed poorer recoveries.

Table 1. In-cell moisture removal of oyster sample using Dionex ASE Prep MAP and Dionex ASE Prep DE, in comparison to sodium sulfate.

Compound	% Recovery Oyster dried with Dionex ASE Prep MAP and Dionex ASE Prep DE* (n = 3)	% Recovery Oyster dried with sodium sulfate** (n = 3)
Lindane	91	81
Heptachlor	93	64
Aldrin	94	66
Dieldrin	105	75
Endrin	106	70
DDT	114	69
Total	101	71

* Data is courtesy of Dr. Todd Anderson from the Department of Toxicology, Texas Tech University, Lubbock

** In-cell drying with sodium sulfate is not recommended using accelerated solvent extraction

Conclusion

This Application Brief describes a simple and reliable method to extract OCPs from oyster tissue. This method also demonstrates the use of Dionex ASE Prep DE and Dionex ASE Prep MAP for in-cell extractions without any pre and post extraction steps to remove moisture and increase extraction efficiencies in wet samples. The method is ideal for routine extractions of OCPs from wet samples.

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Analysis of Multi-Residue Pesticides Present in Ayurvedic Churna by GC-MS/MS

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Keywords: Traditional herbal medicine, fast liquid/liquid extraction, QuEChERS, timed-SRM, retention time synchronization, MRM, ion ratio confirmation, TraceFinder data processing

Introduction

Ayurveda is a Sanskrit term, made up of the words "ayus" and "veda. "meaning life and science; together translating to 'science of life'. A blend of several herbs and spices make up the powdered mixture known as "churna". Depending on its intended use for medicinal, beauty, or culinary purpose, the recipe varies. Avipittakara "churna" is a traditional Ayurvedic formula used widely and almost daily to control vitiated pitta dosha, remove heat in the digestive system, control indigestion, constipation, vomiting and anorexia. A major analytical challenge for these types of samples is mainly addition of multiple herbs with sugar and the natural color of herbs[1].

The dried leaves result in highly complex extracts from the sample preparation due to the rich content of active ingredients, essential oils and the typical high boiling natural polymer compounds. Due to the use of pesticides in the fresh herbs, the "churna" may contain residual pesticides. Analysis of pesticide residues is thus important and governmentally regulated[2]. Strict quality parameters have been mented to preserve the quality and efficacy of these "churnas".

Sample Preparation

In brief, the QuEChERS sample preparation (see Figure 1) involved the extraction of 15 g of a powder sample of Avipittakara "churna" with 15 mL acetonitrile (containing 1% acetic acid) in the presence of 3 g magnesium sulfate, 1.5 g sodium acetate and 1 g NaCl. The supernatant (1 mL) was collected after centrifugation, and dispersive cleanup was performed using 200 mg PSA and 10 mg GCB. The extract was centrifuged at 10 000 rpm for 5 min, and 3 µL of supernatant was

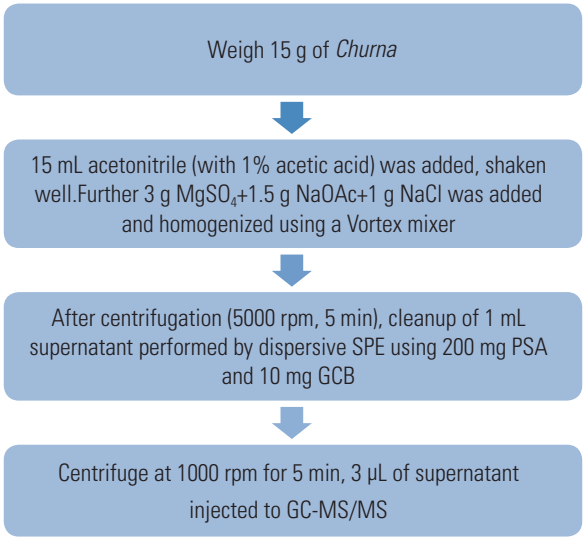

Figure 1. Sample preparation for extraction of pesticides from ayurvedic churnas

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injected via autosampler for analysis. For recovery and validation studies 15 g of the "churna" was fortified with appropriate quantities of the pesticide standard mixture.

Calibration

Stock standard solutions of each pesticide compound were prepared by weighing 10±0.1 mg, dissolving in 10 mL acetonitrile and storing the solution in amber-colored glass vials at -20°C . A total of ten intermediate mixtures (each containing 15-20 compounds) of 10 mg/L concentration were prepared by diluting an adequate quantity of each compound in acetonitrile. A working standard solution (1 mg/L) was prepared by mixing an adequate quantity of intermediate standard solution and dilution with acetonitrile and storing the solution at -20°C . The calibration standards at 2.5, 5, 10, 25 and 50 µg/L were freshly prepared for measurement of the calibration curves. The calibration graphs (five points) for all the compounds were obtained by plotting the individual peak areas against the concentration of the corresponding calibration standards.

Instrument and Method Setup

The analytical method comprises the sample handling using the Thermo Scientific™ TriPlus™ RSH liquid auto sampler, the Thermo Scientific™ TRACE™ 1300 Series gas chromatograph equipped with a temperature programmable PTV injector, and the Thermo Scientific™ TSQ 8000™ triple quadrupole GC-MS/MS system. The instrument method parameters are summarized in Table 1.

TABLE 1. Instrument method parameters.

TRACE™ 1310 Gas Chromatograph Parameters	
Carrier gas	Helium
Injector	PTV
Mode	splitless
Splitless time	3 min, split flow: 30 mL/min
PTV program	87 °C , 0.3 min (injection) 14.5 °C/min to 285 °C (transfer) 285 °C , 2.5 min (transfer) 14.5 °C/min to 290 °C (cleaning) 290 °C , 20 min (cleaning)
Column	Thermo Scientific TraceGOLD™ TG-5 SilMS, 30 m x 0.25 mm x 0.25 µm (p/n 10177894)
Column flow	1.2 mL/min, constant flow
Oven program	70 °C , 2 min 10 °C/min to 200 °C 200 °C , 1 min 10 °C/min to 28 °C 285 °C , 8.5 min
Injection	3 µL by TriPlus RSH Autosampler

The Thermo Scientific™ TraceFinder™ software was used for method setup and data processing. The TraceFinder software provides a compound database of pesticides compoundsof more than 800 compounds with all required analytical details such as retention times and the optimized SRM transitions for data acquisition and processing. These software features were employed to create the processing method for the screening a large pesticides compound list [2].

For all pesticide compounds two SRM transitions were chosen for the overall MRM acquisition method. The first transition was used for quantitation, the second transition for confirmation by checking the ion intensity ratio by the TraceFinder software during data processing. Retention times had been synchronized between data processing of standards with the acquisition method for the timed-SRM protocol (see Figure 2) in order to lock all compound retention times for robustness independent on the impact of the matrix carried by real life sample.

TSQ-8000 MS/MS Parameters	
Ion source temperature	230 °C
Interface temperature	285 °C
Acquisition mode	EI, 70 eV
MRM detection	Timed SRM mode (see Figure 1)
Acquisition rate	500 ms
MRM parameter	See Table 1

The timed-SRM acquisition method used with the TSQ 8000 MS avoids the laborious and time-consuming process of segment creation and method maintenance.The scan times are automatically calculated based upon the specified cycle time so that uniform cycle times are obtained for each mass transition, thus reducing the extensive optimization process for scan times and data points across a peak. The dwell times for data acquisition are maximized independently for the number of compounds in the MRM method. Table 2 lists the MRM parameters for the compounds analyzed in this method.

The data processing and reporting was done using the quantitation and reporting suite. The software allows retention time locking by synchronization between the data processing and the acquisition setup for all compounds in the method.

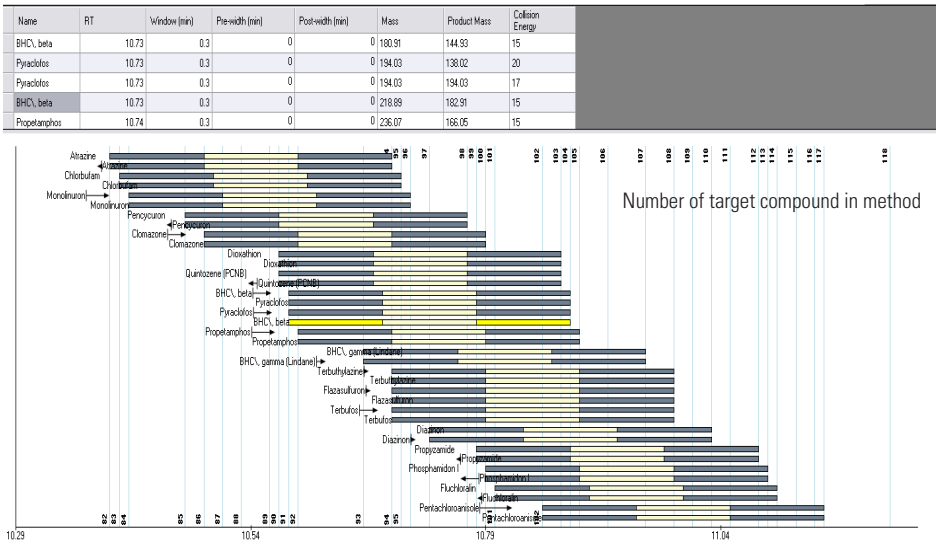

FIGURE 2. Principle of the timed-SRM acquisition setup of the TSQ 8000. The white center parts show the peak width centered to the compound retention time, the grey areas before and after the peak the full SRM acquisition window of 0.3 min.

Results

The multi-residue pesticide analysis of Ayurvedic churnas for routine target analytes detection and quantitationis described using liquid-liquid extraction and GC-MS/MS detection with the TSQ 8000 GC-MS/MS system. All standards and samples were processed using TraceFinder software with high speed and throughput.

All compounds included into this method had very good calibration correlation coefficients of > 0.99 for the concentration range of 2.5 to 50 ng/g, as shown Figure 3. The obtained recoveries were high within 70-120% with < 20% associated RSDs.

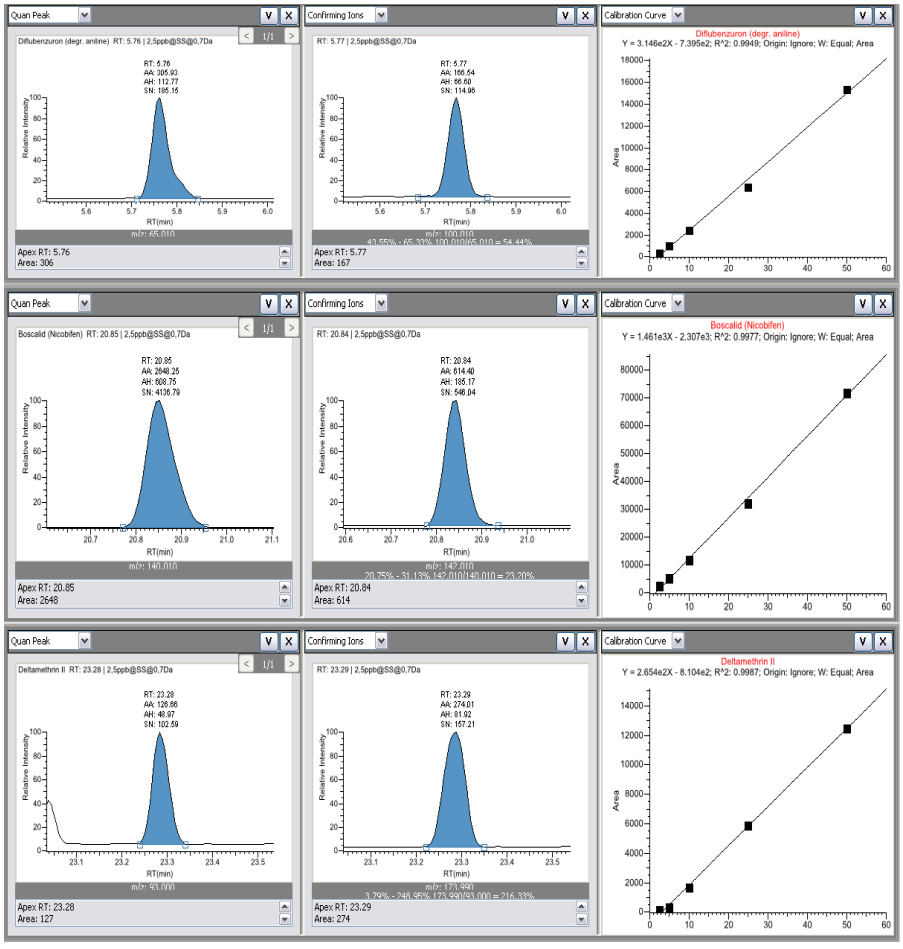

FIGURE 3. Selected pesticide chromatograms at 2.5 ng/g and their calibration curves.

Sample Analysis

Approximately 200 pesticide compounds were included in a routine screening method with an approximately 28 min total run time. The method setup as described above was applied for analyzing samples bought from the regional market.The results from analysis of market samples are presented in Figure 4.

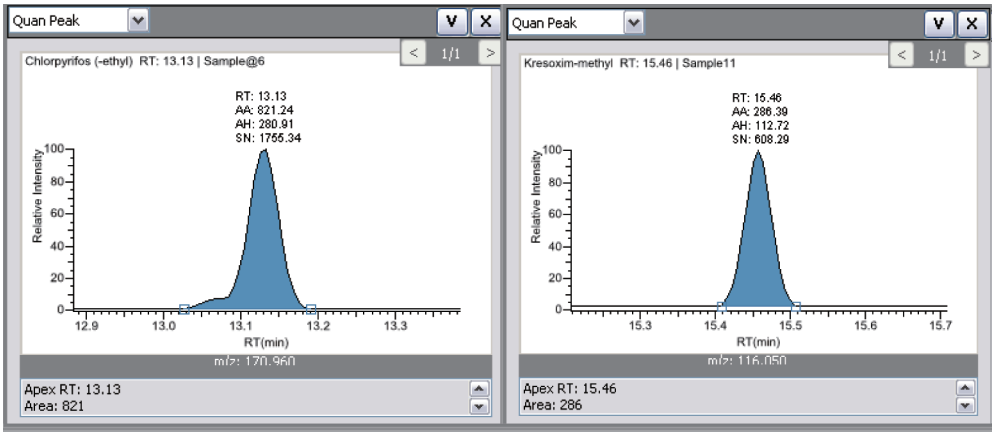

FIGURE 4. Traces of Chlorpyrifos ethyl and Kresoxim methyl were detected at 2.3 and 2.7 µg/kg respectively in regional market samples.

Conclusion

A rapid and sensitive quantitative method for a large number of compounds is always a major goal for analytical laboratories involved in pesticide analysis. Within 28 minutes, 200 pesticides were screened and quantitatively determined using the described pesticide analysis method. The QuEChERS sample preparation method provided high recoveries and good reproducibility. The generic TRACE TR-5MS column coupled with TRACEGuard provided good chromatographic resolution of the pesticides studied. The triple quadrupole mass analyzer TSQ 8000 GC-MS/MS system with TraceFinder™ software was used for data processing to reduce the processing time, thereby resulting in a high throughput method space missing. Linearity, specificity, recovery, and repeatability of the method were established with minimal sample preparation time. The TSQ 8000 system provided very high selectivity for the sensitive detection and reliable quantitation of the pesticides even from these samples with a high matrix load from the short QuEChERS sample preparation.

This method can be utilized for detection and confirmation of trace amounts of pesticides in difficult matrices such as herbal churnas. The method has potential to detect trace level compounds at concentration as low as 2.5 ng/g. As per the available guidelines, the concentration of the detected pesticides (0.0023 and 0.0027 mg/kg) were below the required limits of the Unani Guidelines [3].

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Table 2. MRM parameter for the pesticide compounds analyzed.

Nr.	Compound Name	RT [min]	Quantitation *m/z*	CE [V]	Confirmation *m/z*	CE [V]	R^2
1.	Diflubenzuron (degr. i-cyanat	5.24	153.02 > 90.01	20	153.02 > 125.01	20	0.9969
2.	Diflubenzuron (degr. aniline)	5.75	127.01 > 65.01	30	127.01 > 100.01	30	0.9949
3.	Methamidophos	5.87	141.00 > 95.00	10	141.00 > 126.00	5	0.9930
4.	Dichlorphos (DDVP)	5.94	184.95 > 92.98	17	219.95 > 184.95	10	0.9960
5.	Dichlobenil	6.82	135.97 > 99.98	10	170.96 > 135.97	15	0.9960
6.	Mevinphos	7.39	127.03 > 109.02	10	192.04 > 127.03	12	0.9964
7.	Acephate	7.50	136.01 > 42.00	10	136.01 > 94.01	15	0.9904
8.	Dichloraniline, 3,5-	7.61	160.98 > 89.99	25	160.98 > 98.99	25	0.9989
9.	Molinate (Ordram)	8.58	126.07 > 55.03	10	187.10 > 126.07	10	0.9941
10.	TEPP	8.60	263.06 > 179.04	15	263.06 > 235.06	5	0.9946
11.	Omethoate	9.00	110.01 > 79.01	15	156.02 > 110.01	10	0.9969
12.	Fenobucarb	9.11	121.07 > 77.05	15	150.09 > 121.07	10	0.9977

Nr.	Compound Name	RT [min]	Quantitation *m/z*	CE [V]	Confirmation *m/z*	CE [V]	R^2
13.	Propoxur	9.13	110.06 > 64.03	10	152.08 > 110.06	10	0.9981
14.	Propachlor	9.16	176.06 > 120.04	10	196.07 > 120.04	10	0.9980
15.	Ethoprophos	9.38	158.00 > 80.90	15	158.00 > 114.00	5	0.9949
16.	Trifluralin	9.58	264.09 > 160.05	15	306.10 > 264.09	15	0.9944
17.	Chlorpropham	9.62	213.00 > 127.00	5	213.00 > 171.00	5	0.9981
18.	Benfluralin	9.63	292.10 > 160.05	21	292.10 > 264.09	10	0.9923
19.	Sulfotep	9.70	322.02 > 202.01	15	322.02 > 294.02	10	0.9943
20.	Bendiocarb	9.72	166.06 > 151.06	15	166.06 > 166.06	15	0.9996
21.	Monocrotophos	9.80	127.03 > 95.03	20	127.03 > 109.03	25	0.9971
22.	Methabenzthiazuron	9.82	164.05 > 136.04	12	164.05 > 164.05	10	0.9974
23.	BHC, alpha	10.15	180.91 > 144.93	15	218.89 > 182.91	15	0.9970
24.	Metamitron	10.36	202.09 > 174.07	5	202.09 > 186.08	10	0.9969
25.	Atrazine	10.54	215.09 > 173.08	10	215.09 > 200.09	10	0.9945
26.	Pencycuron	10.62	125.05 > 89.04	12	180.07 > 125.05	12	0.9914
27.	Dioxathion	10.72	125.00 > 97.00	15	125.00 > 141.00	15	0.9936
28.	BHC, beta	10.73	180.91 > 144.93	15	218.89 > 182.91	15	0.9933
29.	Propetamphos	10.74	236.07 > 166.05	15	236.07 > 194.06	5	0.9918
30.	BHC, gamma (Lindane)	10.81	180.91 > 144.93	15	218.89 > 180.91	5	0.9939
31.	Terbuthylazine	10.84	214.10 > 132.06	10	229.11 > 173.08	10	0.9935
32.	Diazinon	10.88	137.05 > 84.03	10	304.10 > 179.06	15	0.9987
33.	Propyzamide	10.93	173.01 > 145.01	15	175.02 > 147.01	15	0.9939
34.	Fluchloralin	10.95	264.04 > 206.03	10	306.05 > 264.04	10	0.9967
35.	Pyroquilon	11.07	173.08 > 130.06	20	173.08 > 145.07	20	0.9974
36.	Pyrimethanil	11.11	198.11 > 158.09	30	198.11 > 183.10	15	0.9953
37.	Tefluthrin	11.16	177.02 > 127.02	20	197.03 > 141.02	15	0.9991
38.	Etrimfos	11.29	292.06 > 153.03	10	292.06 > 181.04	10	0.9935
39.	Pirimicarb	11.50	166.10 > 96.06	10	238.14 > 166.10	15	0.9937
40.	BHC, delta	11.54	180.91 > 144.93	15	204.07 > 91.03	15	0.9949
41.	Iprobenfos	11.54	204.07 > 122.04	15	218.89 > 182.91	15	0.9997
42.	Formothion	11.74	126.00 > 93.00	8	172.00 > 93.00	5	0.9982
43.	Phosphamidon II	11.83	227.05 > 127.03	15	264.06 > 193.04	15	0.9977
44.	Dichlofenthion	11.90	222.98 > 204.98	10	278.97 > 222.98	15	0.9946
45.	Dimethachlor	11.94	197.08 > 148.06	10	199.08 > 148.06	10	0.9992
46.	Dimethenamid	11.95	230.06 > 154.04	10	232.06 > 154.04	10	0.9953
47.	Propazine	12.02	214.09 > 172.08	12	214.09 > 214.09	10	0.9970
48.	Propanil	12.06	217.01 > 161.00	10	219.01 > 163.00	10	0.9934
49.	Malaoxon	12.07	127.02 > 99.02	10	127.02 > 109.02	20	0.9978
50.	Chlorpyrifos-methyl	12.08	124.96 > 78.97	10	285.91 > 92.97	20	0.9945
51.	Metribuzin	12.13	198.08 > 82.03	20	198.08 > 110.05	20	0.9997
52.	Spiroxamine I	12.15	100.09 > 58.05	15	100.09 > 72.06	15	0.9909
53.	Vinclozolin	12.16	212.00 > 172.00	15	285.00 > 212.00	15	0.9957
54.	Carbofuran, 3-Hydroxy	12.21	137.06 > 81.03	18	180.08 > 137.06	15	0.9974
55.	Parathion-methyl	12.22	263.00 > 109.00	15	263.00 > 246.00	15	0.9966
56.	Alachlor	12.23	161.07 > 146.06	12	188.08 > 160.07	10	0.9997
57.	Tolclofos-methyl	12.25	264.96 > 92.99	20	264.96 > 249.96	15	0.9932
58.	Propisochlor	12.31	162.08 > 144.07	10	223.11 > 147.07	10	0.9983
59.	Metalaxyl	12.37	249.13 > 190.10	10	249.13 > 249.13	5	0.9911
60.	Carbaryl	12.41	144.06 > 115.05	20	144.06 > 116.05	20	0.9919
61.	Fuberidazol	12.41	183.80 > 156.10	10	183.80 > 183.10	20	0.9902
62.	Fenchlorfos (Ronnel)	12.47	284.91 > 269.92	13	286.91 > 271.91	20	0.9994
63.	Prosulfocarb	12.63	100.00 > 72.00	10	128.00 > 43.10	5	0.9938
64.	Pirimiphos-methyl	12.66	290.09 > 233.07	10	305.10 > 290.09	15	0.9911
65.	Spiroxamine II	12.75	100.09 > 58.05	15	100.09 > 72.06	15	0.9916
66.	Ethofumesate	12.80	207.08 > 161.06	10	277.02 > 109.01	8	0.9907
67.	Fenitrothion Confirming 1	12.80	277.02 > 260.02	10	286.11 > 207.08	12	0.9997
68.	Methiocarb	12.84	168.06 > 109.04	15	168.06 > 153.06	15	0.9971
69.	Malathion	12.92	127.01 > 99.01	10	173.02 > 127.01	10	0.9951
70.	Dichlofluanid	12.95	223.97 > 122.99	15	225.97 > 122.99	15	0.9971
71.	Phorate sulfone	13.01	153.00 > 125.00	5	199.00 > 143.00	10	0.9942

Nr.	Compound Name	RT [min]	Quantitation *m/z*	CE [V]	Confirmation *m/z*	CE [V]	R^2
72.	Dipropetryn	13.02	241.90 > 149.80	20	254.90 > 180.30	20	0.9906
73.	Chlorpyrifos (-ethyl)	13.12	198.96 > 170.96	15	313.93 > 285.94	12	0.9995
74.	Fenthionoxon	13.22	277.80 > 109.10	25	329.60 > 298.90	10	0.9927
75.	Chlorthal-dimethyl (DCPA)	13.24	300.91 > 300.91	15	331.90 > 300.91	15	0.9986
76.	Flufenacet	13.26	211.04 > 123.02	10	211.04 > 183.03	10	0.9959
77.	Endosulfan I (alpha)	13.43	240.89 > 205.91	20	264.88 > 192.91	22	0.9942
78.	Imazethapyr	13.49	201.9 > 133.00	15	252.00 > 145.90	20	0.9944
79.	Butralin	13.50	266.14 > 190.10	15	266.14 > 220.11	15	0.9996
80.	Pirimiphos (-ethyl)	13.54	304.12 > 168.06	15	333.13 > 318.12	15	0.9992
81.	Pendimethalin	13.86	252.12 > 162.08	12	252.12 > 191.09	12	0.9912
82.	Fipronil	13.87	212.97 > 177.98	16	366.95 > 212.97	25	0.9938
83.	Cyprodinil	13.91	224.13 > 208.12	20	225.13 > 210.12	18	0.9959
84.	Metazachlor	13.92	133.05 > 117.04	20	209.07 > 132.05	12	0.9939
85.	Penconazole	14.01	248.06 > 157.04	25	248.06 > 192.04	15	0.9977
86.	Tolylfluanid	14.05	137.05 > 91.03	20	238.09 > 137.05	15	0.9922
87.	Chlorfenvinphos-Z	14.05	266.98 > 158.99	15	322.97 > 266.98	15	0.9904
88.	Allethrin	14.06	123.08 > 81.05	10	136.08 > 93.06	10	0.9923
89.	Mecarbam	14.09	226.04 > 198.03	5	329.05 > 160.03	10	0.9979
90.	Phenthoate	14.18	146.01 > 118.01	10	274.03 > 246.02	10	0.9951
91.	Mephosfolan	14.20	196.02 > 140.02	15	196.02 > 168.02	10	0.9973
92.	Quinalphos	14.21	146.03 > 118.02	15	157.03 > 129.02	13	0.9943
93.	Triflumizole	14.31	179.04 > 144.04	15	206.05 > 179.04	15	0.9925
94.	Procymidone	14.31	283.02 > 96.01	15	283.02 > 255.02	10	0.9983
95.	Bromophos-ethyl	14.50	358.89 > 302.91	20	358.89 > 330.90	10	0.9985
96.	Methidathion	14.60	124.98 > 98.99	22	144.98 > 84.99	10	0.9945
97.	Chlordane, alpha (cis)	14.62	372.81 > 265.87	18	374.81 > 267.87	15	0.9967
98.	DDE, o,p	14.63	245.95 > 175.97	25	317.94 > 245.95	20	0.9946
99.	Sulfallate	14.68	188.02 > 132.02	22	188.02 > 160.02	16	0.9945
100.	Paclobutrazol	14.72	236.10 > 125.06	15	236.10 > 167.07	15	0.9926
101.	Disulfoton sulfone	14.74	213.01 > 125.01	10	213.01 > 153.01	5	0.9912
102.	Picoxystrobin	14.77	303.09 > 157.04	20	335.09 > 303.09	10	0.9937
103.	Endosulfan II (beta)	14.88	271.88 > 236.89	18	338.85 > 265.88	15	0.9973
104.	Mepanipyrim	14.89	222.11 > 207.10	15	223.11 > 208.10	15	0.9965
105.	Chlordane, gamma (trans)	14.89	372.81 > 265.87	18	374.81 > 267.87	15	0.9991
106.	Flutriafol	14.97	123.04 > 75.03	15	219.07 > 123.04	15	0.9915
107.	Napropamide	15.00	128.07 > 72.04	10	271.16 > 128.07	5	0.9972
108.	Flutolanil	15.03	173.06 > 145.05	15	173.06 > 173.06	15	0.9988
109.	Pretilachlor	15.13	162.09 > 147.08	15	216.05 > 174.04	20	0.9935
110.	Hexaconazole, confirming 1	15.13	231.06 > 175.04	10	262.14 > 202.11	15	0.9962
111.	Isoprothiolane	15.14	290.06 > 118.03	15	290.06 > 204.05	15	0.9961
112.	Profenofos	15.21	138.98 > 96.98	8	338.94 > 268.95	20	0.9939
113.	Oxadiazon	15.26	258.05 > 175.04	10	304.06 > 260.05	10	0.9927
114.	DDE, p,p	15.32	245.95 > 175.97	25	317.94 > 245.95	20	0.9964
115.	Myclobutanil	15.40	179.07 > 125.05	15	179.07 > 152.06	15	0.9912
116.	Buprofezin	15.43	172.09 > 57.03	10	249.13 > 193.10	10	0.9906
117.	Kresoxim-methyl	15.44	206.09 > 116.05	15	206.09 > 131.06	15	0.9921
118.	DDT, o,p'	15.47	234.94 > 164.96	15	234.97 > 164.98	20	0.9935
119.	DDT, o,p', confirming 1	15.47	236.94 > 164.96	20	236.97 > 164.98	20	0.9963
120.	Aramite-1	15.48	185.06 > 63.02	15	319.10 > 185.06	15	0.9959
121.	Aramite-2	15.69	185.06 > 63.02	15	319.10 > 185.06	15	0.9971
122.	Carpropamid	15.78	139.00 > 103.10	10	222.00 > 125.00	18	0.9982
123.	Cyproconazole	15.79	222.09 > 125.05	20	224.09 > 127.05	20	0.9989
124.	Nitrofen	15.85	201.99 > 138.99	21	282.98 > 252.98	15	0.9997
125.	Chlorobenzilate	15.98	251.02 > 139.01	20	253.03 > 141.01	15	0.9978
126.	Oxadiargyl	15.99	149.90 > 122.90	15	285.00 > 255.00	14	0.9963
127.	Fenthion sulfoxide	16.05	279.01 > 153.01	15	294.02 > 279.01	8	0.9958
128.	Diniconazole	16.11	268.06 > 232.05	15	270.06 > 234.05	15	0.9949
129.	Ethion	16.12	230.99 > 202.99	15	383.99 > 230.99	10	0.9973
130.	Oxadixyl	16.16	132.06 > 117.05	15	163.07 > 132.06	10	0.9985

Nr.	Compound Name	RT [min]	Quantitation *m/z*	CE [V]	Confirmation *m/z*	CE [V]	R^2
131.	DDT, p,p'	16.20	234.94 > 164.96	20	234.94 > 164.96	20	0.9979
132.	DDD, p,p'	16.20	234.97 > 164.98	20	236.97 > 164.98	20	0.9959
133.	Chlorthiophos1	16.20	324.96 > 268.97	15	324.96 > 296.97	10	0.9969
134.	Imiprothrin	16.36	123.00 > 81.00	5	324.90 > 269.20	14	0.9967
135.	Mepronil	16.45	269.14 > 119.06	10	269.14 > 210.11	10	0.9945
136.	Triazophos	16.46	161.03 > 134.03	10	257.05 > 162.03	10	0.9936
137.	Ofurace	16.58	186.05 > 158.05	10	232.07 > 186.05	10	0.9973
138.	Carfentrazone-ethyl	16.59	330.03 > 310.03	20	340.03 > 312.03	10	0.9919
139.	Benalaxyl	16.63	234.12 > 174.09	10	266.14 > 148.08	10	0.9951
140.	Trifloxystrobin	16.65	116.04 > 89.03	15	190.06 > 130.04	10	0.9962
141.	Propiconazole, peak 1	16.77	259.02 > 69.01	20	259.02 > 173.02	20	0.9989
142.	Edifenphos	16.78	173.01 > 109.01	15	310.03 > 173.01	10	0.9904
143.	Quinoxyfen	16.84	272.00 > 237.00	20	307.00 > 272.00	10	0.9982
144.	Endosulfan sulfate	16.85	271.88 > 236.89	15	273.88 > 238.89	15	0.9929
145.	Clodinafop-propargyl	16.87	349.05 > 238.04	15	349.05 > 266.04	15	0.9991
146.	Flupicolide	16.90	208.80 > 182.00	20	261.00 > 175.00	24	0.9988
147.	Hexazinone	17.02	171.00 > 71.00	10	171.00 > 85.00	10	0.9998
148.	Propargite	17.16	135.06 > 107.05	15	350.16 > 201.09	10	0.9991
149.	Diflufenican	17.21	266.05 > 246.05	10	394.07 > 266.05	10	0.9981
150.	Triphenylphosphate (TPP)	17.26	325.07 > 169.04	25	326.07 > 325.07	10	0.9995
151.	Iprodione	17.65	187.02 > 124.01	20	187.02 > 159.02	40	0.9979
152.	Bifenthrin	17.77	181.05 > 153.05	6	181.05 > 166.05	15	0.9922
153.	Picolinafen	17.90	376.08 > 238.05	15	376.08 > 239.05	15	0.9981
154.	Bromopropylate	17.91	184.98 > 156.98	20	342.96 > 184.98	20	0.9967
155.	Fenoxycarb	17.93	186.08 > 186.08	10	255.11 > 186.08	10	0.9933
156.	Fenpropathrin	18.01	181.09 > 152.07	23	265.13 > 210.10	15	0.9956
157.	Fenamidone	18.10	238.08 > 237.08	20	268.09 > 180.06	20	0.9994
158.	Tebufenpyrad	18.11	276.13 > 171.08	15	333.16 > 276.13	10	0.9997
159.	Fenazaquin	18.23	145.08 > 117.07	15	160.09 > 117.07	20	0.9951
160.	Imazalil	18.25	173.03 > 145.02	20	215.04 > 173.03	15	0.9954
161.	Furathiocarb	18.27	163.07 > 107.04	10	325.13 > 194.08	10	0.9989
162.	Flurtamone	18.38	199.06 > 157.05	20	333.10 > 120.04	15	0.9945
163.	Tetradifon	18.46	226.93 > 198.94	18	353.88 > 158.95	15	0.9973
164.	Phosalone	18.54	181.99 > 111.00	15	181.99 > 138.00	10	0.9985
165.	Triticonazole	18.57	217.09 > 182.07	10	235.10 > 217.09	10	0.9945
166.	Pyriproxyfen	18.68	136.06 > 78.03	15	136.06 > 96.04	15	0.9941
167.	Cyhalofop butyl	18.70	256.10 > 120.05	10	256.10 > 256.10	10	0.9969
168.	Tralkoxydim	18.80	137.00 > 57.20	10	181.04 > 152.03	23	0.9995
169.	Cyhalothrin, lambda	18.80	197.04 > 141.03	15	234.90 > 217.20	15	0.9997
170.	Lactofen	18.83	344.04 > 223.02	15	344.04 > 300.03	15	0.9975
171.	Benfuracarb	19.03	164.08 > 149.07	10	190.09 > 144.07	10	0.9975
172.	Pyrazophos	19.05	221.05 > 193.04	10	232.05 > 204.05	10	0.9930
173.	Fenarimol	19.15	139.01 > 111.01	15	219.02 > 107.01	15	0.9993
174.	Azinphos-ethyl	19.20	132.01 > 77.01	20	160.02 > 132.01	5	0.9944
175.	Fenoxaprop-P	19.41	288.03 > 260.03	10	361.04 > 288.03	10	0.9998
176.	Bitertanol1	19.59	170.09 > 115.06	25	170.09 > 141.07	20	0.9993
177.	Permethrin, peak 1	19.68	183.04 > 165.03	15	183.04 > 168.03	15	0.9973
178.	Bitertanol2	19.71	170.09 > 115.06	25	170.09 > 141.07	20	0.9993
179.	Permethrin, peak 2	19.81	183.04 > 165.03	15	183.04 > 168.03	15	0.9909
180.	Prochloraz	19.88	180.01 > 138.01	15	310.03 > 268.02	10	0.9932
181.	Cafenstrole	20.21	100.04 > 72.03	15	188.08 > 119.05	15	0.9991
182.	Cyfluthrin, peak 1	20.26	163.02 > 91.01	12	163.02 > 127.02	10	0.9915
183.	Fenbuconazole	20.34	129.04 > 102.03	15	198.07 > 129.04	10	0.9996
184.	Cypermethrin I	20.65	163.03 > 127.02	10	181.03 > 152.03	25	0.9996
185.	Boscalid (Nicobifen)	20.84	342.03 > 140.01	15	344.03 > 142.01	15	0.9977
186.	Flucythrinate, peak 1	20.85	199.07 > 107.04	22	199.07 > 157.06	10	0.9958
187.	Quizalofop-Ethyl	20.92	299.07 > 255.06	20	372.09 > 299.07	15	0.9969
188.	Etofenprox	21.08	163.09 > 107.06	16	163.09 > 135.07	10	0.9987
189.	Flucythrinate, peak 2	21.12	199.07 > 107.04	22	199.07 > 157.06	10	0.9989

Nr.	Compound Name	RT [min]	Quantitation *m/z*	CE [V]	Confirmation *m/z*	CE [V]	R^2
190.	Fenvalerate, peak 1	21.94	167.05 > 125.04	10	419.13 > 225.07	10	0.9978
191.	Fluvalinate, peak 1	22.09	250.06 > 200.05	20	252.06 > 200.05	20	0.9973
192.	Pyraclostrobin	22.17	132.03 > 77.02	15	325.08 > 132.03	20	0.9936
193.	Fluvalinate, peak 2	22.20	250.06 > 200.05	20	252.06 > 200.05	20	0.9977
194.	Fenvalerate, peak 2	22.28	167.05 > 125.04	10	419.13 > 225.07	10	0.9996
195.	Difenoconazole, peak 1	22.76	323.05 > 265.04	15	325.05 > 267.04	20	0.9995
196.	Indoxacarb	22.95	203.03 > 106.01	20	203.03 > 134.02	20	0.9996
197.	Deltamethrin II	23.28	252.99 > 93.00	18	252.99 > 173.99	18	0.9987
198.	Azoxystrobin	23.63	344.10 > 329.10	20	388.11 > 345.10	15	0.9991
199.	Dimethomorph-1	23.91	301.10 > 165.05	10	387.12 > 301.10	12	0.9992
200.	Dimethomorph-2	24.60	301.10 > 165.05	10	387.12 > 301.10	12	0.9990

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Application Note 20549

QuEChERS Dispersive Solid Phase Extraction for the GC-MS Analysis of Pesticides in Cucumber

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Key Words
QuEChERS, pesticide residue analysis, cucumber, food safety

Abstract
QuEChERS dispersive SPE is a simple, fast and quantitative sample preparation method. This application demonstrates the effectiveness of this technique in the GC/MS analysis of pesticides in cucumber, using a Thermo Scientific TraceGOLD TG-5MS GC column for analysis.

The recoveries for the spiked pesticides in cucumber matrix at 50 ng/g were between 75.2 to 119.6% with relative standard deviations ranging from 2.1 to 8.9% using the QuEChERS method described in EN15662.

Introduction
QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) is a dispersive Solid Phase Extraction (SPE) technique for extracting multi-residue pesticides from fruits and vegetables. The advantages of this methodology are speed, ease of execution, minimal solvent requirement and cost. The QuEChERS methodology was developed by Anastassiades et al[1] and has become widely used in food safety analyses.

The method is:

- **Quick** – high sample throughput, typically 8 samples can be prepared in under 30 min
- **Easy** – it requires less handling of extracts than other techniques i.e. fewer steps are required
- **Cheap** – less sorbent material is needed and less time is required to process samples compared to other techniques
- **Effective** – the simple technique gives high and accurate recovery levels for a range of different compound types.
- **Rugged** – the method can detect a large number of pesticides including charged and polar pesticides
- **Safe** – unlike other techniques, it does not require the use of chlorinated solvents. Extraction is typically carried out using acetonitrile, which is both GC and LC compatible.

The sample preparation approach described in the European EN15662 QuEChERS procedure[2] was used for extracting pesticides from cucumber. This is a two stage process: sample extraction, followed by dispersive SPE.

In the sample extraction stage, the food sample is homogenized to increase the available surface area of the sample to provide optimal extraction efficiencies. The homogenized sample is placed in the extraction tube containing magnesium sulfate and salts (sodium chloride, sodium citrate tribasic dihydrate, sodium citrate dibasic sesquihydrate). Magnesium sulfate ensures that, upon addition of acetonitrile, a phase separation is induced between water and organic solvent with the pesticides of interest being extracted into the organic phase. When acetonitrile is poured into the extraction tube containing the homogenized sample,

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an exothermic reaction occurs between the magnesium sulfate and water. This step may lead to reduced recoveries of the pesticides. To overcome this problem, the sample can be weighed directly into an empty centrifuge tube followed by the addition of acetonitrile.The tube can then be immersed in an ice bath with slow addition of salts.

The second stage of the QuEChERS method uses dispersive SPE, which involves transferring a portion of the acetonitrile extract to a clean-up tube containing a combination of sorbents for removal of unwanted sample components. The sample clean-up also reduces matrix effects and therefore improves method robustness.

The pesticides analyzed in the cucumber matrix include mixtures of herbicides, fungicides, organophosphorus pesticides and pyrethroids. Six extractions of 50 ng/g spiked level were used for the recovery experiments.

Experimental Details

Consumables		Part Number
Column:	TraceGOLD TG-5MS, 30 m x 0.25 mm x 0.25 µm	26098-1420
Septum:	BTO, 17 mm	31303211
Liner:	Splitless Straight Liner, 3 x 8 x 105 mm	45350033
Column ferrules:	100% Graphite ferrules for TRACE injector 0.1-0.25 mm ID	29053488
	Graphite/vespel for transfer line 0.1-0.25 mm ID	29033496
Injection syringe:	10 µL Fixed needle syringe for a TriPlus Autosampler	36500525
Thermo Scientific Chromacol 9mm screw 2 mL vial -clear		2-SVW
Thermo Scientific Chromacol 9mm screw caps with PTFE/Silicone/PTFE		9-SC(B)-TST1

Chemicals and Reagents		Part Number
QuEChERS Extraction Stage:	Metalized Pouch containing 6g $MgSO_4$, 1.5 g sodium chloride, 1.5 g sodium citrate tribasic dihydrate, 0.75 g sodium citrate dibasic sesquihydrate and empty centrifuge tube with plug seal cap	60105-337
QuEChERS Dispersive SPE Stage:	15 mL centrifuge tube with 900 mg $MgSO_4$, 150 mg PSA, 150 mg C18	60105-227
Fisher Scientific HPLC grade Acetonitrile	A/0626/17	

Sample Preparation

The methodology described in this application note is for the preparation of calibration standards and sample spike (Figure 1).

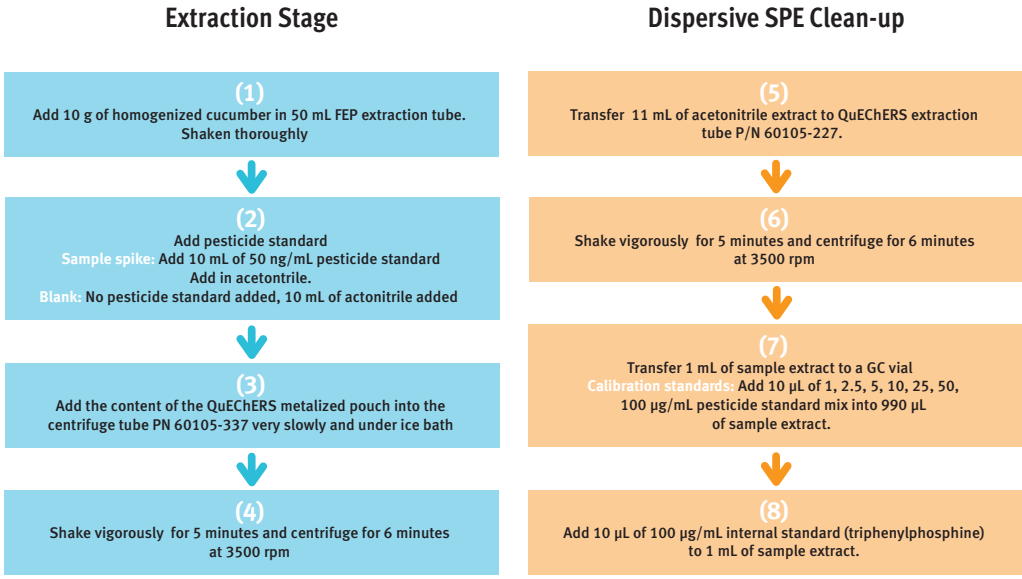

Figure 1: Flow diagram of QuEChERS methodology used in this application

Separation Conditions	Part Number
Instrumentation:	Thermo Scientific TRACE GC Ultra
Carrier gas:	Helium
Column flow:	1.0 mL/min, Constant flow
Oven temperature:	90 °C (1 min), 30 °C/min, 180 °C (0.5 min), 5 °C/min, 280 °C (5 min), 30 °C/min, 320 °C (10 min)
Injector type:	Programmable Temperature Vaporizer (PTV)
Injector mode:	PTV Splitless (0.75 min)
Injector conditions:	80 °C (0.05 min), evaporation 14.5 °C/sec, 180 °C (1 min), transfer 2.5 °C/sec, 300 °C (3 min), 14.5 °C/sec, 330 °C (20 min) 50 mL/min flow rate

MS Conditions	
Instrumentation:	Thermo Scientific ISQ Single Quadrupole mass spectrometer
Transfer line temperature:	282 °C
Source temperature:	280 °C
Ionization conditions:	EI
Electron energy:	70 eV

Segment	Compound	Start time (min)	m/z (Quan) Qual ions	Dwell time/ sec
1	Dichlobenil	3.50	(171), 173, 100, 136	0.05
2	Tribromoanisol	6.20	(346), 344, 329, 331	0.05
3	Sulfotep	6.80	(322), 97, 202, 146	0.05
4	Hexachlorobenzene	7.40	(284), 282, 283, 214	0.05
5	Parathion	10.40	(291), 109, 97	0.05
6	Triphenylphosphine (IS)	13.00	(262), 183, 108	0.05
7	EPN	18.90	(157), 169, 141, 110	0.05
8	Azinphos methyl	19.00	(160), 77, 132	0.05
9	Permethrin	20.90	(183), 163, 165, 153	0.05
10	Fenvalerate	23.00	(125), 167, 225	0.05
11	Deltamethrin	25.60	(253), 181, 251, 152	0.05

Table 1: SIM Scan Parameters

Injection Conditions	
Instrumentation:	Thermo Scientific TriPlus Autosampler
Injection Volume:	2 µL

Data Processing	
Software:	Software: Thermo Scientific XCalibur™

Results

In order to assess the method linearity, a calibration curve was constructed for each of the ten pesticides spiked in the sample matrix, using triphenylphosphine as the internal standard (IS). The concentration range studied for the selected pesticides in Table 2 was 25 to 1000 ng/g. The coefficient of determination (R^2) between area ratio of sample and internal standard for all pesticides were higher than 0.99 (Table 2), demonstrating good method linearity.

The analysis was performed in SIM. Figure 2 shows the TIC chromatogram of spiked pesticides in cucumber matrix at 10 µg/g in full scan. Six extractions of ten pesticides in sample matrix spiked at 50 ng/g were measured. These values do not appear to correlate with those in table 2.

The recoveries for spiked pesticides were between 75 and 120%, with an relative standard deviation (RSD) under 9% (Table 2).

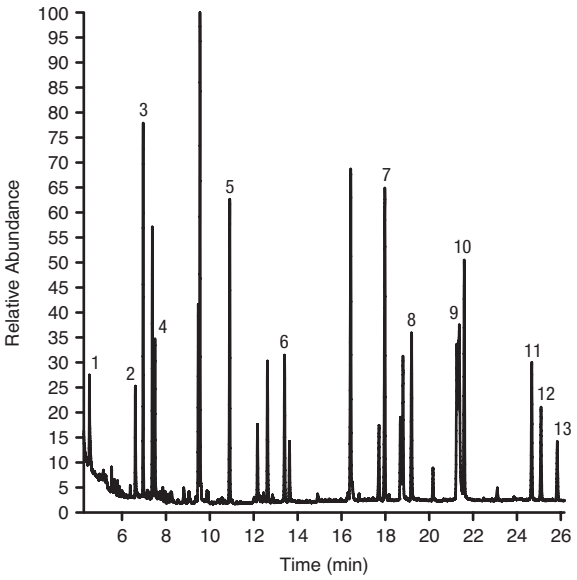

Figure 2: TIC for the GC/MS analysis of cucumber matrix spiked with 10 µg/g of each pesticide in full scan (50-450 m/z). See Table 2 for identified peaks. Unidentified peaks are matrix peaks

Conclusion

The QuEChERS sample preparation method provided high recoveries and good reproducibility. The QuEChERS – GC/MS method was found to be linear in the concentration range of 25 to 1000 ng/g spiked matrix. The TraceGOLD TG-5MS GC column provided good chromatographic resolution of the pesticides studied.

References

1. M. Anastassiades, S.J. Lehotay, D. Stajnbaher and F.J. Schenck, J AOAC Int 86 (2003) 412.

2. Foods of plant origin - Determination of pesticide residues using GC/MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE –QuEChERS method. European Standard EN15662:2008

Pesticides	t_R (min)	Linearity	Nominal concentration ng/g	Measured concentration (n=6) ng/g.	Average % Recovery (n=6)	Recovery %RSD (n=6)
1. Dichlobenil	4.52	0.9988	50	58.7	117.4	2.1
2. Tribromoanisol	6.60	0.9990	50	54.3	108.5	6.0
3. Sulfotep	6.95	0.9984	50	59.8	119.6	2.3
4. Hexachlorobenzene	7.49	0.9983	50	55.2	110.4	2.8
5. Parathion	10.90	0.9979	50	53.0	106.0	5.9
6. Triphenylphosphine (IS)	13.41	-	-	-	-	-
7. EPN	17.90	0.9985	50	46.1	92.1	6.7
8. Azinphos methyl	19.20	0.9984	50	37.6	75.2	4.9
9. Permethrin isomer a	21.38	0.9987	50	49.8	99.5	8.9
10. Permethrin isomer b	21.58	0.9985	50	50.9	101.9	4.8
11. Fenvalerate isomer a	24.60	0.9985	50	47.7	95.4	8.9
12. Fenvalerate isomer b	25.02	0.9973	50	50.6	101.2	7.2
13. Deltamethrin	25.84	0.9949	50	51.7	103.3	6.8

Table 2: Summary of Results
Unidentified peaks in Figure 2 are impurity/matrix peaks

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The Importance of Autosampler Vial Selection in the GC-MS Analysis of Pyrethroid Pesticides at Low Concentration

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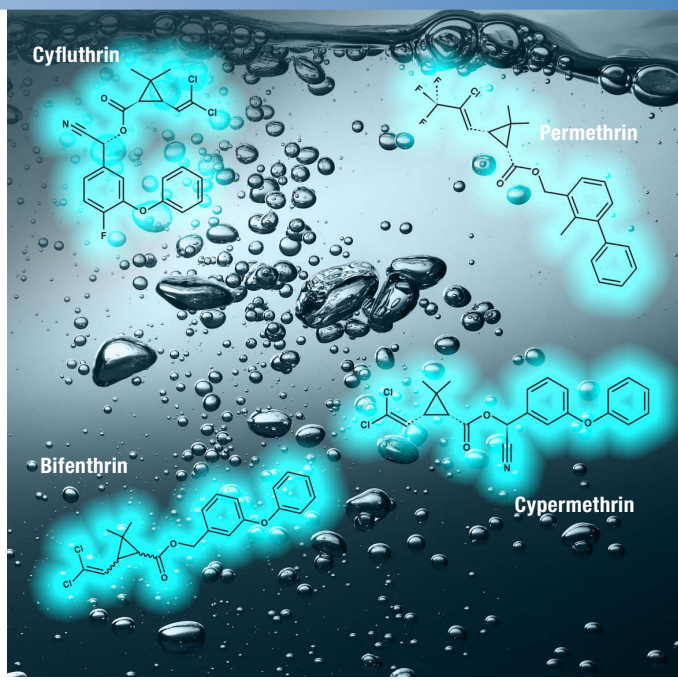
Application Note 20642

Key Words

Pyrethroid pesticides, adsorption, 33 expansion high purity clear neutral borosilicate glass vial

Abstract

The selection of the correct autosampler vial type is vital to the success of analyzing pyrethroid pesticides at low levels. A method for the determination of pyrethroids at 0.10 ng/mL was developed using solid phase extraction (SPE) for pre-concentration with subsequent analysis by GC with PTV simulated on-column injection. Careful selection of the autosampler vial type was needed to minimize adsorption effects.



Introduction

Pyrethroids are a class of synthetically produced insecticides that are mainly used for domestic purposes to control insects such as house flies and mosquitoes. They behave very similarly to natural pyrethrins, which are derived from chrysanthemum flowers, and are extremely toxic to fish and aquatic organisms but have low toxicity towards humans. However, repeated exposure to pyrethroids increases the risk of anaphylaxis and allergic reaction to very low concentrations and, therefore, pyrethroid levels should be monitored.

Analyzing pyrethroids at low concentration levels can be challenging due to their adsorption onto glass surfaces, such as sample bottles, GC inlet liners and vials. To reduce adsorption of pyrethroids onto the surface of glass, a comparison with plastic and high purity clear neutral borosilicate glass vials was carried out with a GC method utilizing a Programmable Temperature Vaporizer (PTV) simulated on-column injection.

The separation of the pyrethroid extracts was carried out using a Thermo Scientific™ TraceGOLD™ TG-5SilMS column with a Thermo Scientific GuardGOLD™ pre-column. This column is based on silarylene chemistry, which provides more stability and lower bleed than standard 5% phenyl dimethylpolylsiloxane phase GC columns. This in turn gives rise to better sensitivity due to reduced background signal. This phase can also partially resolve complex mixtures of cyfluthrin and cypermethrin isomers.

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Experimental Details

Consumables		Part Number
Columns:	TraceGOLD TG-5SilMS, 30 m x 0.25 mm x 0.25 µm GuardGOLD 2 m x 0.53 mm ID Press-Fit	26096-1420 26050-0253 64000-001
Injection port septum:	BTO, 12.7 mm	313032280
Liner:	PTV Silcosteel liner for PTV simulated on-column, 1 x 2.75 x 120 mm	45322052
Column ferrules:	100% graphite ferrules for Thermo Scientific TRACE™ injector, 0.53 mm ID	29053486
Colum ferrules:	Graphite/Vespel® for transfer line, 0.25 mm ID	29033496
Injection syringe:	85 mm 26s Gauge, 10 µL fixed needle syringe for a Thermo Scientific TriPlus™ RSH Autosampler	365D0321
Sample vials:	Thermo Scientific National™ 9 mm Target DP Polypropylene Vial, 300 µL	C4000-11
	Thermo Scientific National 9 mm Target DP™ Vial, Total Recovery with 10 µL Reservoir	C4000-9TR
	Thermo Scientific Chromacol™ 9 mm screw caps with High Purity Silicone/PTFE septa	9-SC(B)-ST101
	LC-MS grade water	

Preparation of Calibration Standards

A stock standard solution of 1 mg/mL of bifenthrin, permethrin, cyfluthrin, and cypermethrin was prepared in ethyl acetate. Calibration standard solutions were then prepared in ethyl acetate at the following concentrations: 50, 100, 200, 500, 1000, and 2000 ng/mL. A 100 µL aliquot of each calibration standard was then placed into an autosampler vial followed by the addition of 10 µL of 10 µg/mL of internal standard to each vial.

Sample Preparation: SPE Extraction Protocol		Part Number
SPE cartridge:	Thermo Scientific HyperSep™ C18 SPE column, 2000 mg/15 mL	60108-701
Compound:	(i) Bifenthrin, (ii) *cis/trans* permethrin, (iii) cyfluthrin, and (iv) cypermethrin 1 L each at 0.10 ng/mL in water	
Matrix:	LC/MS water	
Conditioning stage:	10 mL ethyl acetate, 10 mL acetone, 2 x 10 mL aliquots water applied sequentially to the SPE cartridge and then pulled through under vacuum at 4-5 mL/min	
Application stage:	1 L of sample was applied to the SPE cartridge under vacuum at 4-5 mL/min	
Washing stage:	10 mL of water was added to a 1 L vessel, swirled, and placed onto the SPE cartridge. Then the cartridge was dried for 20 min under vacuum.	
Elution stage:	10 mL ethyl acetate was added to the sample vessel, swirled, and then placed onto the SPE cartridge. Then an additional 10 mL ethyl acetate was applied directly onto the cartridge.	
Additional stages:	Solvent was evaporated to dryness at 40 °C and the residue reconstituted in 100 µL of ethyl acetate to give the final concentration of pyrethroids at 1000 ng/mL. Then 10 µL of 10 µg/mL of internal standard was added to the vial.	

Separation Conditions	
Instrumentation:	Thermo Scientific TRACE GC Ultra™
Carrier gas:	Helium
Split flow:	50 mL/min
Column flow:	1.2 mL/min, Constant flow
Oven temperature:	80 °C (0.5 min), 30 °C/min, 220 °C (4 min), 10 °C/min, 320 °C (10 min)
Injector type:	PTV simulated on-column
Injector mode:	Splitless (10 min) 30 mL/min flow rate, constant septum purge
Injector temperature phases:	40 °C (0.10 min), transfer 12 °C/sec, 330 °C (10 min) min
Detector type:	Thermo Scientific ISQ™ mass spectrometer
Transfer line temperature:	260 °C
Source temperature:	220 °C
Ionization conditions:	EI
Electron energy: 70	eV
Emission current:	25 µA
SIM scan parameters:	Table 1

Scan Window Start Time (min)	Compound Name	Mass List (Quan), Qual ions	Total Scan Time (s)
5.50	1,2,3,4-tetrachloro-naphthalene (IS)	(264), 268, 266,	0.222
9.50	Bifenthrin	(181), 165, 166, 182	0.216
14.00	*cis/trans* permethrin	(183), 184, 163, 165	0.216
15.40	Cyfluthrin/cypermethrin	(226), (181), 182, 163, 165, 166	0.234

Table 1: SIM Scan parameters

Injection Conditions	
Instrumentation:	Thermo Scientific TriPlus RSH Autosampler
Injection Volume:	2 µL
Injection depth:	70 mm
Penetration speed:	10 mm/s
Injection speed:	50 µL/s

Results

Due to the possible breakdown of pyrethroids if exposed to light during sample preparation, standard solutions were initially prepared in amber glass vials. Investigation showed that the choice of a non-silanized 51A amber (type 1, class B) glass autosampler vial was impacting the recovery of the compounds from the vials. The results showed that the overall recovery of the pyrethroids was poor and the calibration of both bifenthrin and permethrin showed non-linear response (Figure 1). The higher levels of iron oxide present in the amber vials used as a coloring agent leaches out when in contact with water. The glass surface then becomes more active and interacts with pyrethroids.

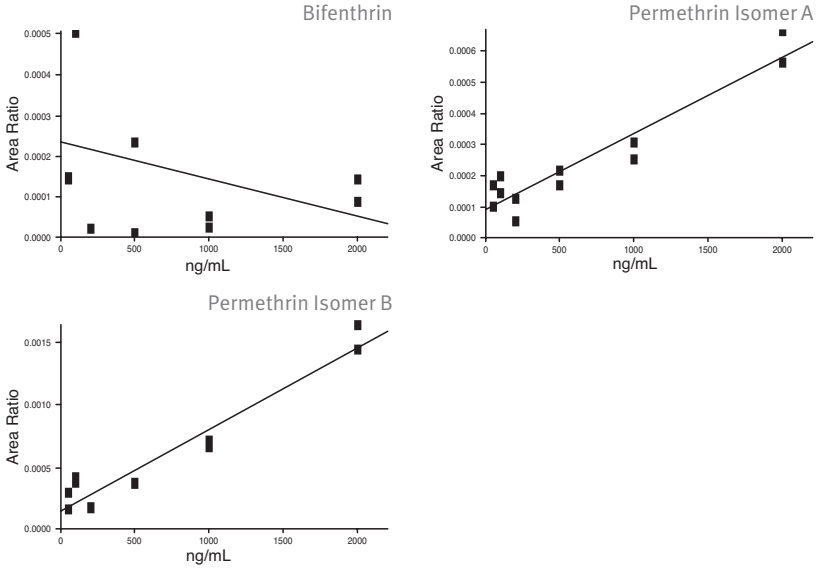

Figure 1: Calibration curves for bifenthrin and permethrin isomers (50–2000 ng/mL) using non-silanized amber glass vial

To determine if the glass vial was responsible for adsorption of pyrethroids, it was decided to substitute the non-silanized amber vials with an alternative plastic vial. The results obtained showed improved recovery and linearity of sample response (Figure 2, Table 2). However, the plastic vials, composed of polypropylene, would also be likely to introduce leachable organic species when exposed to the organic solvent for any extended period of time. The contact time between the solvent and plastic vials had to be kept to a minimum to avoid introduction of these polypropylene extractables into the mass spectrometer.

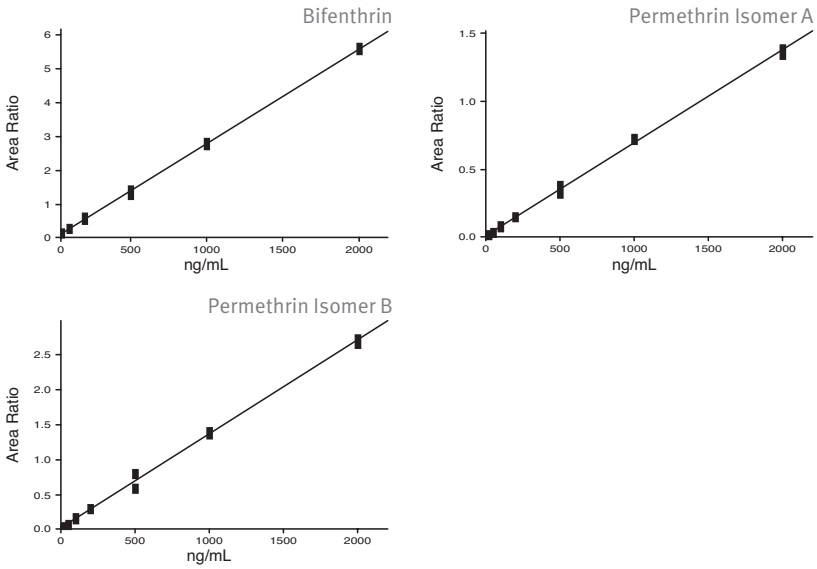

Figure 2: Calibration curves for bifenthrin and permethrin isomers (50–2000 ng/mL) using a plastic vial

To determine if the type of glass could have an effect on adsorption of pyrethroids, a high purity clear neutral borosilicate glass vial was evaluated. Thermo Scientific National 9 mm Target DP Total Recovery Vials, 33 expansion borosilicate clear (Type 1, Class A) were used. This gave improved linearity and extraction recoveries for all pyrethroids (Figure 3, Table 2). In this case, the contact time was not found to be a limiting factor, and the pyrethroid samples could be safely stored in the vial, if shielded from direct sunlight.

Vials	Plastic Vial			Target DP 33 Expansion High Purity Clear Neutral Borosilicate Glass Vial			Non-Silanized Amber Glass Vial		
Compound	Linearity R^2	% Recovery	% RSD (n=3)	Linearity R^2	% Recovery	% RSD (n=3)	Linearity R^2	% Recovery	% RSD (n=3)
Bifenthrin	0.9995	81.8	7.2	0.9988	101.8	4.0	0.1426	49.5	–
Permethrin Isomer a	0.9984	85.7	12.1	0.9975	117.1	4.4	0.9016	46.33	–
Permethethrin Isomer b	0.9979	85.6	5.7	0.9978	112.2	3.9	0.9296	8.08	–
Cyfluthrin Total Isomers	0.9953	101.6	6.0	0.9977	117.1	3.6	0.9967	62.61	–
Cypermethrin Total Isomers	0.9957	84.7	4.5	0.9963	113.9	4.2	0.9744	62.61	–

Table 2: Comparison data of linearity (50–2000 ng/mL) and extraction recovery for 0.10 ng/mL spiked pyrethroids in water using plastic, 33 expansion high purity clear neutral borosilicate glass, and non-silanized amber vials.

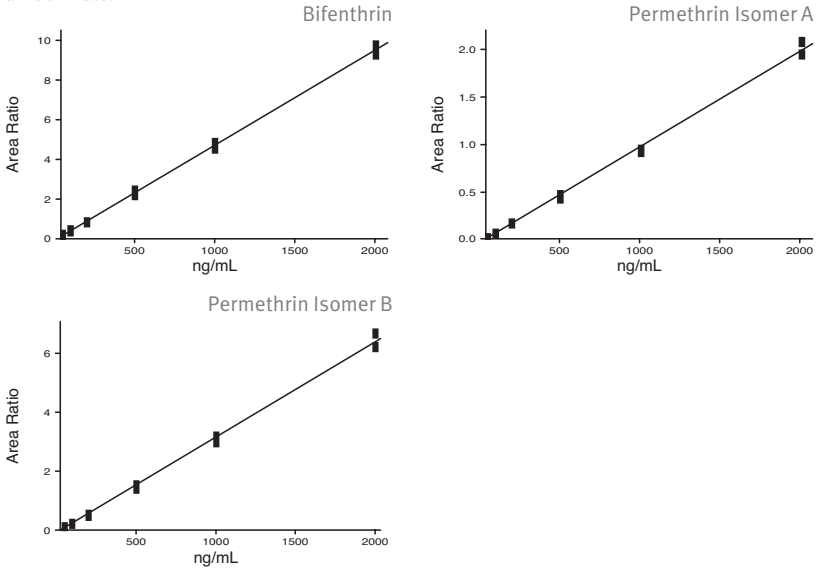

Figure 3: Calibration curves for bifenthrin and permethrin isomers (50–2000 ng/mL) using a 33 expansion high purity clear neutral borosilicate glass vial

A calibration curve (50-2000 ng/mL) was constructed for each compound using 1,2,3,4-tetrachloronaphthalene as the internal standard (IS). The coefficients of determination (R^2) between area ratio of sample and internal standard for all pyrethroids were greater than 0.99 for plastic and Target DP Total Recovery Vials, 33 expansion borosilicate clear (Type 1, Class A) (Table 2), demonstrating good method linearity. The analysis was performed in SIM mode. Figure 4 shows the TIC chromatogram of spiked pyrethroids in water at 0.10 ng/mL after the pre-concentration step.

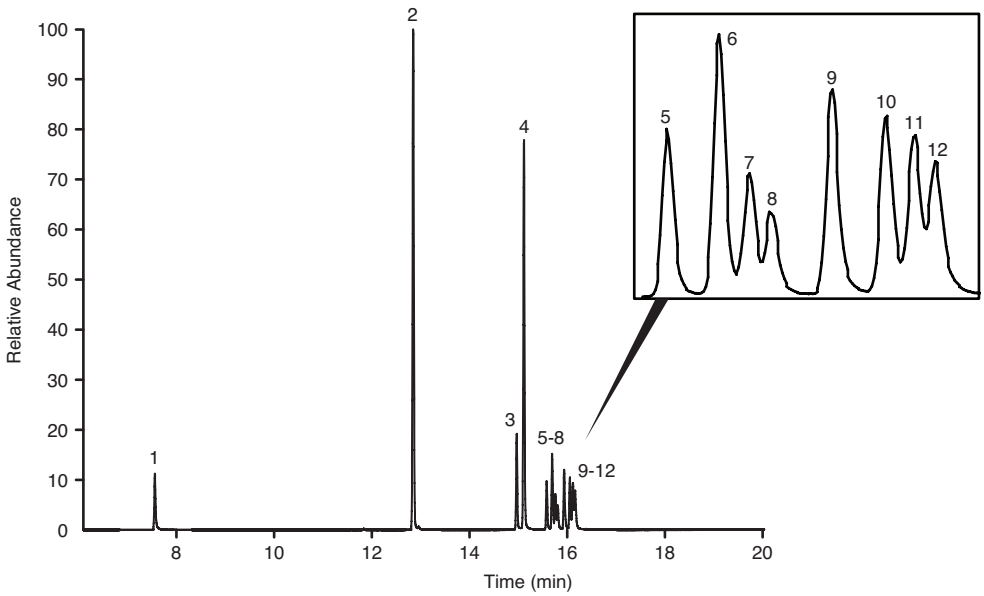

Figure 4: SIM Chromatogram of 0.10 ng/mL of pyrethroid pesticides separated on TG-5SilMS column after a pre-concentration step using a HyperSep C18 SPE cartridge

Peak Number	Compound	t_R (min)
1	1,2,3,4-Tetra Chloronaphthalene (IS)	7.56
2	Bifenthrin	12.84
3	Permethrin isomer a	14.97
4	Permethrin isomer b	15.11
5	Cyfluthrin isomer a	15.58
6	Cyfluthrin isomer b	15.69
7	Cyfluthrin isomer c	15.76
8	Cyfluthrin isomer d	15.81
9	Cypermethrin isomer a	15.94
10	Cypermethrin isomer b	16.06
11	Cypermethrin isomer c	16.12
12	Cypermethrin isomer d	16.16

Table 3: Peak indentification

Three replicate extractions of pyrethroids spiked at 0.10 ng/mL in water were carried out using a HyperSep C18 SPE cartridge. The extraction recoveries from plastic, non-silanized amber vials, and Target DP Total Recovery Vials, 33 expansion borosilicate clear (Type 1, Class A), were compared. The pyrethroids recoveries were measured to be 81%–117%, with relative standard deviations (RSD) of 3%–12% (see Table 2 for individual pyrethroids measured at each concentration). The extraction recovery was enhanced when using the high purity 33 expansion glass vials as shown in Figure 5.

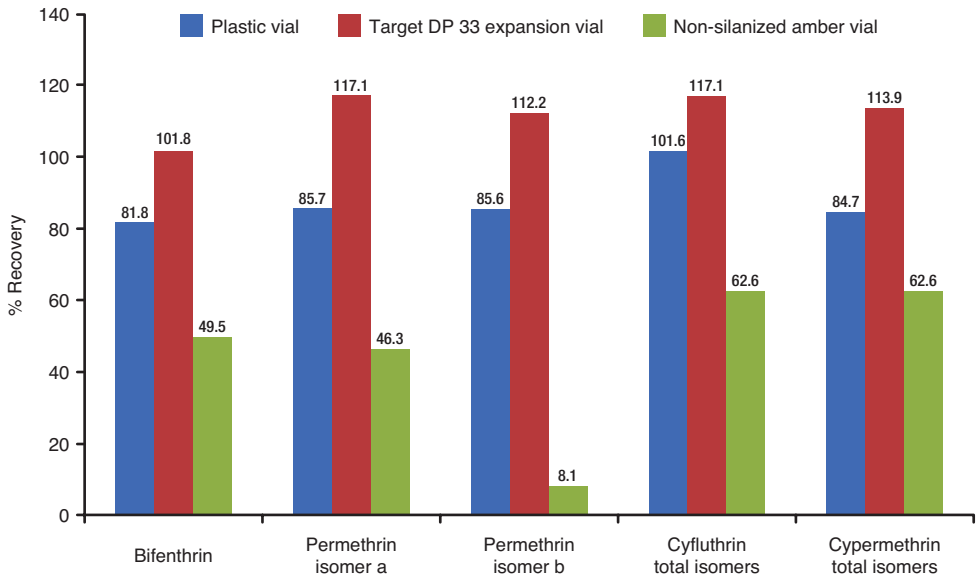

Figure 5: Comparison of extraction recoveries of pyrethroids using three different vial types

Conclusion

- Studies showed plastic and high purity clear neutral borosilicate glass vials reduce adsorption of pyrethroids at lower concentrations.
- The linearity and recovery of the pyrethroids was improved by the use of polypropylene vials but at the risk of absorbed material being introduced into the GC.
- The best results were obtained using a high purity 33 expansion clear glass vial with low surface activity, which gave higher sample recovery compared to the polypropylene vial.
- The SPE-GC/MS method demonstrated high recovery for 0.10 ng/mL of pyrethroids in water.
- The GC/MS method was found to be linear over the range of 50 to 2000 ng/mL.
- The HyperSep C18 silica SPE cartridge allowed the extraction and preconcentration of pyrethroids in water for quantification.

References

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3. ISO 719:1985 Glass – Hydrolytic resistance of glass grains at 98 degrees C – Method of test and classification.

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Application Note 20705

Analysis of Organophosphorus Pesticides by GC

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Key Words

Organophosphorus pesticides, TraceGOLD TG-5MS column, TRACE 1310, US EPA Method 8141B, quartz liner

Abstract

This application note demonstrates the use of a deactivated, splitless quartz liner with single taper and a 5% phenyl polysiloxane phase column for the separation of an organophosphorus pesticides standard mix. This was analyzed on a Thermo Scientific™ TRACE™ 1310 GC equipped with a modular split/splitless (SSL) injector and a flame ionization detector (FID).

Introduction

US EPA 8141B is one of a number of standard analytical methods used for the determination of organophosphorus pesticides (OPPs) in aqueous and solid samples by gas chromatography. OPP can easily degrade in the injector port, which can lead to poor peak profiles. This causes activity within the GC inlet port when repeated injections are made, producing matrix effects. These pesticides can then interact with the active sites and produce peak tailing and poor reproducibility of results.

Using a Thermo Scientific deactivated, packed splitless quartz liner results in a reduction of activity on the surface of the liner, giving excellent reproducibility when compared to several other liner formats. The liner is treated using a proprietary process to reduce any surface activity. These characteristics lead to highly symmetrical peak shapes. In addition, deactivated quartz wool helps in trapping the non-volatile compounds.

This analysis is performed on an ultra-low bleed 5% phenylpolysiloxane phase GC column. The OPP analysis was performed in splitless injection mode using a Thermo Scientific™ TraceGOLD™ TG-5MS 30 m × 0.25 mm × 0.25 µm GC column and a deactivated, packed splitless quartz wool liner for the TRACE 1310 GC, which is equipped with a modular plug and play split/splitless (SSL) injector and a flame ionization detector (FID). This fulfills the requirement of US EPA Method 8141B for the analysis of the OPPs listed in Table 1.

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Consumables		Part Number
Column:	TraceGOLD TG-5MS 30 m × 0.25 mm × 0.25 µm	26098-1420
Septum:	BTO coated 11 mm center guide (50/pk)	31303233
Liner:	Splitless liner with single taper 78.5 × 4 × 6.3 mm	453A1925
Column ferrules:	Graphite ferrule for 0.1–0.32 mm i.d. columns 10/pk	290GA139
Injection syringe:	10 µL syringe FN 50 mm T Gauge 26, cone tip	36500525
Vials and closures:	Thermo Scientific 9 mm Wide Opening Screw Thread Vials Convenience Kit, 2 mL Clear Glass Vial with ID Patch, Blue Closure with PTFE/Blue Silicone Septa	60180-599

Solutions
A working standard solution of 20 µg/mL of EPA 8141 was prepared in acetone. The stock solution was obtained commercially at a concentration of 1000 µg/mL.

Separation Conditions		Part Number
Instrumentation:	TRACE 1310 mainframe 230 V GC	14800302
Carrier gas:	Helium	
Split flow:	50 mL/min	
Column flow:	1.2 mL/min, constant flow	
Oven temperature:	40 °C (1 min), 12 °C/min, 280 °C (10 min)	
Injector type:	TRACE 1310 SSL Injector module	29903010
Injector mode:	Splitless	
Injection details:	Splitless (1 min)	
Injector temperature:	220 °C	
Detector details:	TRACE 1310 FID module	29903001
FID parameters:		
Temperature:	280 °C	
Air flow:	350 mL/min	
Hydrogen flow:	35 mL/min	
Nitrogen makeup flow:	30 mL/min	

Injector Conditions	
Instrumentation:	Thermo Scientific AS1300 Autosampler
Injection Volume:	1 µL
Wash solvent:	Acetone/hexane (1:1 v/v)

Data Processing	
Software:	Thermo Scientific™ Chrom-Card™ data system

Results

Figure 1 shows the TIC chromatogram for 22 OPPs at 20 µg/µL using a TraceGOLD TG-5MS column and a standard, deactivated, splitless quartz liner for the TRACE 1310 GC instrument. Table 1 shows the peak identification of the OPPs according to their retention times. Table 1 includes the reproducibility data for ten injections. The stationary phase of the TG-5MS GC column, in combination with the deactivated splitless liner, provides excellent performance due to minimal interaction of active compounds with active sites on the column, the glass wall of the liner, or the deactivated quartz wool. This minimizes peak tailing of the OPPs and gives highly symmetrical peak shapes. The combination of a TG-5MS GC column, the deactivated liner, and the TRACE 1310 GC gave excellent injection reproducibility of between 1.7% and 3.4% for the 22 OPPs tested (Table 2).

The tailing factors calculated according to the USP method for all peaks were 0.82–0.97 apart from mevinphos, which gave a tailing factor of 0.77. The resolution value between peaks 17 and 18 was 1.75 according to the USP criteria. For peaks 12 and 13, the calculated resolution was 0.90.

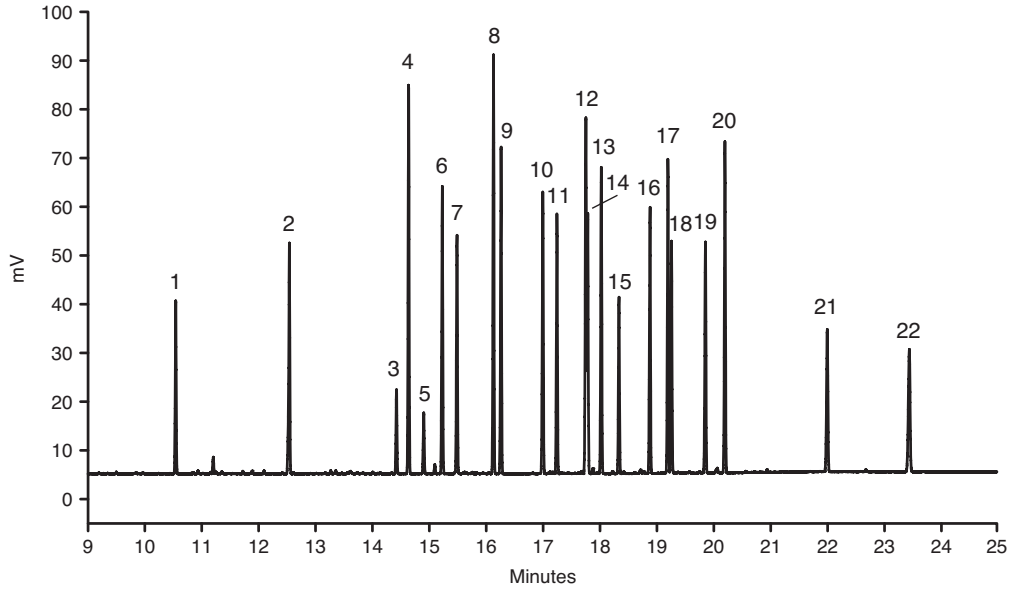

Figure 1: Chromatogram of 22 OPP standards at 20 µg/mL

Peak Number	Compound	t_R (min)	t_R %RSD (n=10)	Peak Area %RSD (n=10)
1	Dichlorvos	10.55	0.02	1.8
2	Mevinphos	12.55	0.02	2.0
3	Demeton O	14.43	0.02	2.6
4	Ethroprophos	14.65	0.01	2.0
5	Naled	14.91	0.01	2.6
6	Phorate	15.24	0.01	1.8
7	Demeton S	15.50	0.01	1.9
8	Diazinon	16.14	0.01	1.9
9	Disulfoton	16.27	0.01	1.7
10	Methyl parathion	17.01	0.01	2.2
11	Fenchlorphos	17.26	0.01	2.0
12	Fenthion	17.77	0.02	2.3
13	Chlorpyrifos	17.80	0.02	3.4
14	Trichloronate	18.04	0.01	1.9
15	Merphos	18.35	0.01	1.9
16	Stirofos	18.90	0.01	2.0
17	Tokuthion	19.21	0.02	2.1
18	Impurity	19.27	0.01	2.2
19	Fensulfothion	19.87	0.01	2.1
20	Bolstar	20.22	0.01	2.0
21	Azinphos methyl	22.02	0.01	2.3
22	Coumaphos	23.46	0.01	2.1

Table 1: List of OPPs and their retention times peak area reproducibility

Conclusion

The TraceGOLD TG-5MS column and the deactivated, splitless quartz liner with quartz wool, when used in a TRACE 1310 GC instrument, demonstrated excellent performance for the separation and analysis of organophosphorus compounds with excellent peak shape, resolution, and reproducibility.

Reference

US EPA 8141B: http://water.epa.gov/scitech/methods/cwa/index.cfm

Acknowledgements

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AN20705_E 03/13S

Application Note 52279

High Precision Pesticide Analysis in Produce using GC Triple Quadrupole and U-SRM Mode

Inge de Dobbeleer, Joachim Gummersbach, Hans-Joachim Huebschmann, Anton Mayer,
Thermo Fisher Scientific, Dreieich, Germany

Key Words
1 ppb Levels, Challenging Compounds, Pesticide Analysis, PTV Backflush, U-SRM Mode, Selected Reaction Monitoring, Selectivity, Timed-SRM

Introduction

Pesticides are widely used in agriculture to protect crops and to improve efficiency of production. Consequently, governments, food producers and food retailers have the duty to ensure that any residues occurring in foods for human consumption are at or below Statutory Maximum Residue Levels (MRLs). Regulation EC 396/2005 adopted in the European Union sets MRLs for more than 500 different pesticides in over 300 different food commodities.[1]

Many of these MRLs are set at a default value of 0.01 mg/kg, the typical limit of determination of routine analytical methods. Thus, there is a requirement for food safety laboratories to test a wide array of foods for a large number of pesticide residues at concentrations at or below 0.01 mg/kg, with low costs and fast turnaround times (often <48 hours). For the efficient control of the regulated MRL levels, the overall method sensitivity in matrix is required to be a factor of 10 lower. This is most often achieved using multi-residue methods based on the use of a combination of LC-MS/MS and GC-MS techniques to determine pesticide residues in a single generic solvent extract of the sample. One such example is the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) procedure, which is based on acetonitrile extraction and dispersive solid phase extraction.[2] After the QuEChERS extraction, a solvent exchange was made to facilitate the GC injection.

The productivity benefit of using the QuEChERS extraction technique is the fast turnaround time for a large number of samples with small sample volumes in the range of 10 g. Limitations of this approach are typically arising from the heavy matrix load of QuEChERS extract requiring increased robustness of the GC inlet system and increased selectivity offered by using a MS/MS analyzer. This application note describes the high quality and low level analysis of pesticides in produce samples using the Thermo Scientific TSQ Quantum XLS Ultra GC-MS/MS system.

For most of the pesticide compounds included in the method, the complete list of the compounds with their respective SRM transitions have been downloaded from the Pesticides Method Reference CD (provided with the manual p/n 120390) into the instrument acquisition method. Each transition has been determined for optimal sensitivity and selectivity, with the complete list documented for TSQ Quantum XLS users.

Over 400 pesticides have been monitored in several matrices such as wheat, blackcurrants and cucumber; the results of the most challenging pesticides in terms of activity and response are highlighted, showing calibration curves, repeatability and ion ratio stabilities.

The TSQ Quantum XLS Ultra™ is able to perform SRM with a higher mass resolution (0.1 Da) setting thus allowing for better selectivity. Not all pesticides in all matrices benefit from a higher mass resolution setting, but depending on the matrix and the compound analyzed, there can be a significant improvement on the signal to noise ratio. Some examples are shown in the 'Advanced GC-MS/MS Experiment' section of this application note.

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Experimental Conditions

All samples were prepared using the QuEChERS technique, and calibration was performed using a blank QuEChERS extract from cucumber. All target compounds were measured using at least two SRM transitions for each compound to a level of 0.001 mg/kg, which is ten times lower than the current maximum concentration limit.

All sample analyses were carried out using the TSQ Quantum XLS Ultra GC-MS/MS system, equipped with a Thermo Scientific TRACE GC Ultra gas chromatograph.

The TRACE GC Ultra™ was configured with a B.E.S.T. PTV injector equipped with a backflush device. Sample introduction was performed using the Thermo Scientific TriPlus RSH autosampler. The capillary column was a Thermo Scientific TraceGOLD TG-5MS column (5% phenyl film) of 30 m length, 0.25 mm inner diameter and 0.25 µm film thickness (Table 1).

The pre-column used was a 1.2 m TG-5HT, 0.15 µm film thickness and 0.53 mm inner diameter (see Table 1).

TRACE GC Ultra	
Injection Volume	2 µL injection
Liner	Siltec® baffled liner (part number 453T2120)
Carrier Gas	He, constant flow, 1.3 mL/min
Column Type	TraceGOLD™ TG-5MS column (5% phenyl film) of 30 m length, 0.25 mm inner diameter and 0.25 µm film thickness (part number 26098-1420)
Precolumn	1.2 m of TraceGOLD TG-5HT column of 30 m length, 0.53 mm inner diameter and 0.15 µm film thickness (part number 26095-0620)
GC Method	Initial 65 °C, Hold 1.5 min, Ramp 30.0 °C/min–150 °C, Ramp 5.0 °C/min–290 °C, Ramp 30.0 °C/min–320 °C, Hold 5.0 min
Transfer Line	300 °C
TRACE GC Ultra PTV Program	
Injector Temperature	70 °C, splitless injection 1.5 min
PTV Inject	70 °C, 0.2 min, 8 °C/sec to transfer step
PTV Transfer	280 °C, 21 min, 10 °C/sec to clean step
PTV Clean	350 °C, 33 min, clean flow 30 mL/min
Transfer Time	21 min
TSQ Quantum XLS Ultra Mass Spectrometer	
Source Temperature	240 °C, CEI volume
Ionization	EI, 70 eV
Emission Current	50 µA
Resolution	0.7 Da Q1, Q3; 0.1 Da on Q1, 0.7 on Q3 for the wheat examples
Collision Gas	Argon, 1.5 mTorr

Table 1: Selected instrument conditions for the employed TRACE GC Ultra and TSQ Quantum XLS Ultra mass spectrometer

Maximizing Robustness

High boiling compounds in sample matrix have a negative effect on the analytical column's quality and lifetime, requiring a bake out process at high temperatures, thus limiting sample throughput. A backflush process was used to protect the column, allowing more samples to be injected before the phase attachment on the surface of the column becomes weak. Being able to inject more samples before necessary column replacement improves throughput and reduces costs per analyses.

During backflushing of the pre-column, the injector was set to a higher temperature and increased flow. This also allowed the injector liner to be swept of residual matrix contaminants during analysis time. This concurrent backflush operation results in the complete system staying clean and inert for a high number of injections, resulting in less maintenance frequencies.[3]

Method Setup

The method parameters for the PTV concurrent backflush operation, GC separation and TSQ Quantum XLS Ultra mass spectrometer setup are given in Table 1.

Each compound SRM transition was only monitored for a narrow time window around the established retention time (timed SRM). This led to a fully optimized instrument duty cycle for maximum analytical performance being handled automatically by the system. The complete list can be copied into the instrument method, thus saving time and avoiding entry errors.[4]

For data acquisition, the two most selective transitions were chosen after reviewing data from spiked matrix samples. Selection criteria were based on the absence of interferences from the matrix, along with signal generation of the transition.

Results and Discussion

Advanced GC-MS/MS Experiments – U-SRM

The patented Thermo Scientific HyperQuad technology in the TSQ Quantum XLS Ultra system offered high sensitivity by high ion transmission already found at the standard nominal mass resolution settings (0.7 Da FWHM). In addition, the HyperQuad™ technology allows the possibility to enhance the applied mass resolution for increased selectivity during analysis. The significantly increased selectivity further reduces the background caused by matrix components, thus giving a cleaner peak detection and high signal-to-noise results.

Some compound transitions are more susceptible to matrix interference than others. Standard SRM resolution (0.7 Da) can often provide enough selectivity to overcome most matrix interference challenges. In complex matrices, however, even with the structure-selective SRM acquisitions, removal of the isobaric matrix interference is insufficient.

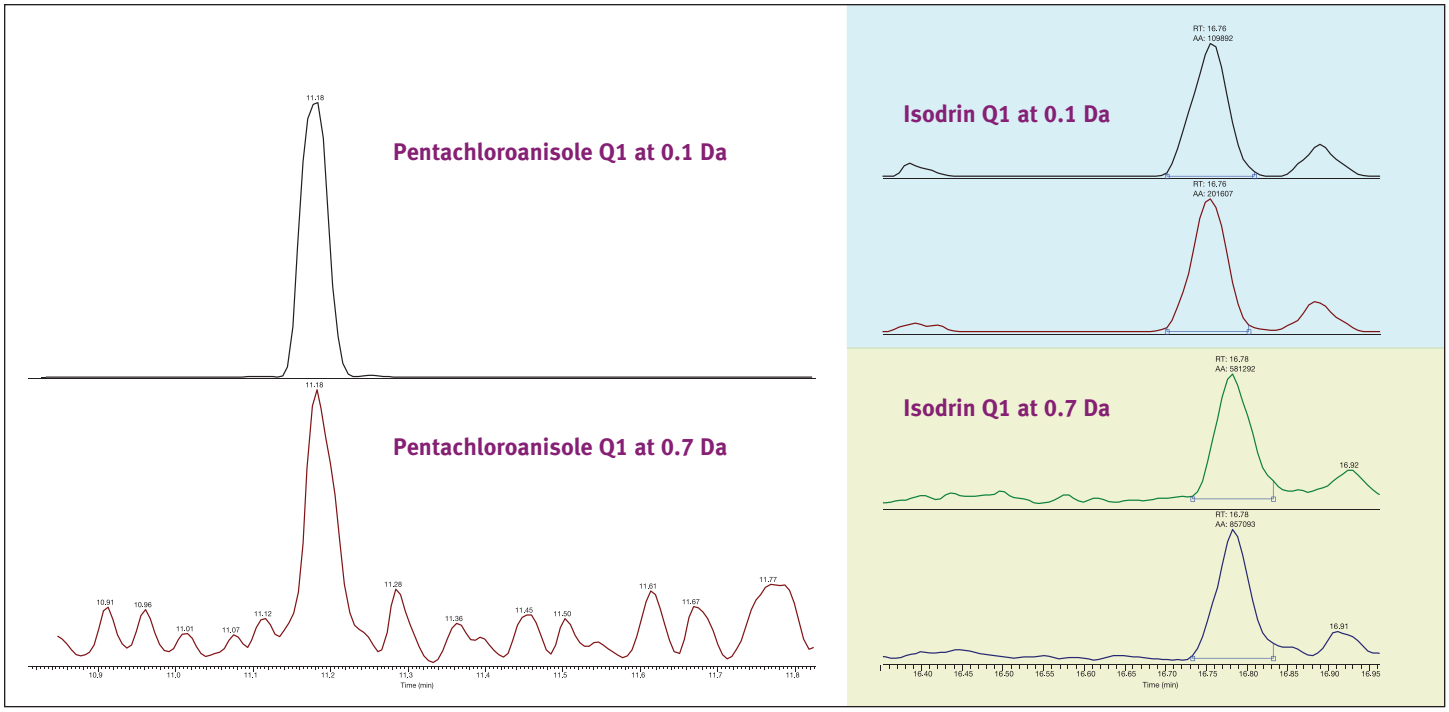


Figure 1: Comparison of U-SRM and standard SRM for pentachloroanisole and isodrin in wheat at 10 ppb levels;
Top: The chromatogram in U-SRM SRM (Q1 FWHM at 0.1 Da); Bottom: The same sample in standard mode (Q1 FWHM at 0.7 Da).

By increasing the mass resolution (down to 0.1 Da) of the first quadrupole during SRM acquisitions, a more selective isolation of the compound pre-cursor ion is achieved. This acquisition mode is known as Ultra-Selective Reaction Monitoring (U-SRM).

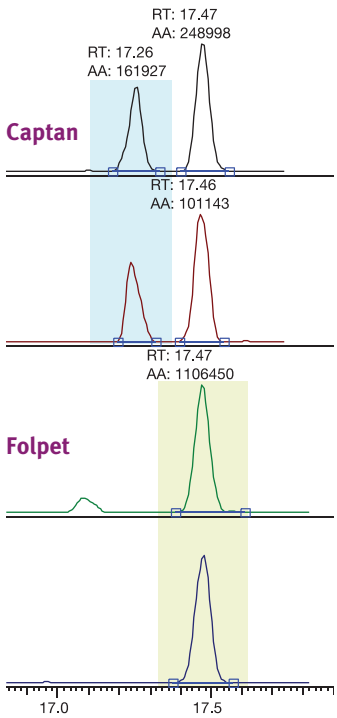
Figure 1 gives examples of U-SRM acquisition of pentachloroanisole and isodrin at 10 ppb in wheat matrix.

Analytical Performance

The complete method validation was performed using standard mass resolution settings at 0.7 Da.

A very comfortable detection of virtually all pesticides was achieved at the 1 ppb level. Excellent linearity was also observed with correlation values exceeding 0.995 for the linear calibration. In addition to this, the residual errors for each calibration point were less than 10% for all compounds (RSD). This included a calibration point at the 1 ppb level.

Also, more difficult compounds such as Captan and Folpet showed excellent peak signal and repeatability when using this method.



Injections (10 ppb blackcurrant)	Captan (area)	Folpet (area)
1	221347	247021
2	180365	229513
3	196336	273992
4	189745	277547
5	199317	273831
6	176386	270323
7	174745	296082
8	203117	231265
9	210897	248086
10	231017	234245
11	193543	264083
12	208722	292804
13	184633	246250
14	175942	285853
15	204764	295590
Average	196725.1	264432.3
Standard Deviation	16988.97	23534.93
RSD	**8.64%**	**8.90%**

Figure 2 and Table 2: Captan (RT 17.26) and Folpet (RT 17.47) in blackcurrant extract spiked at 10 ppb level, showing both transitions

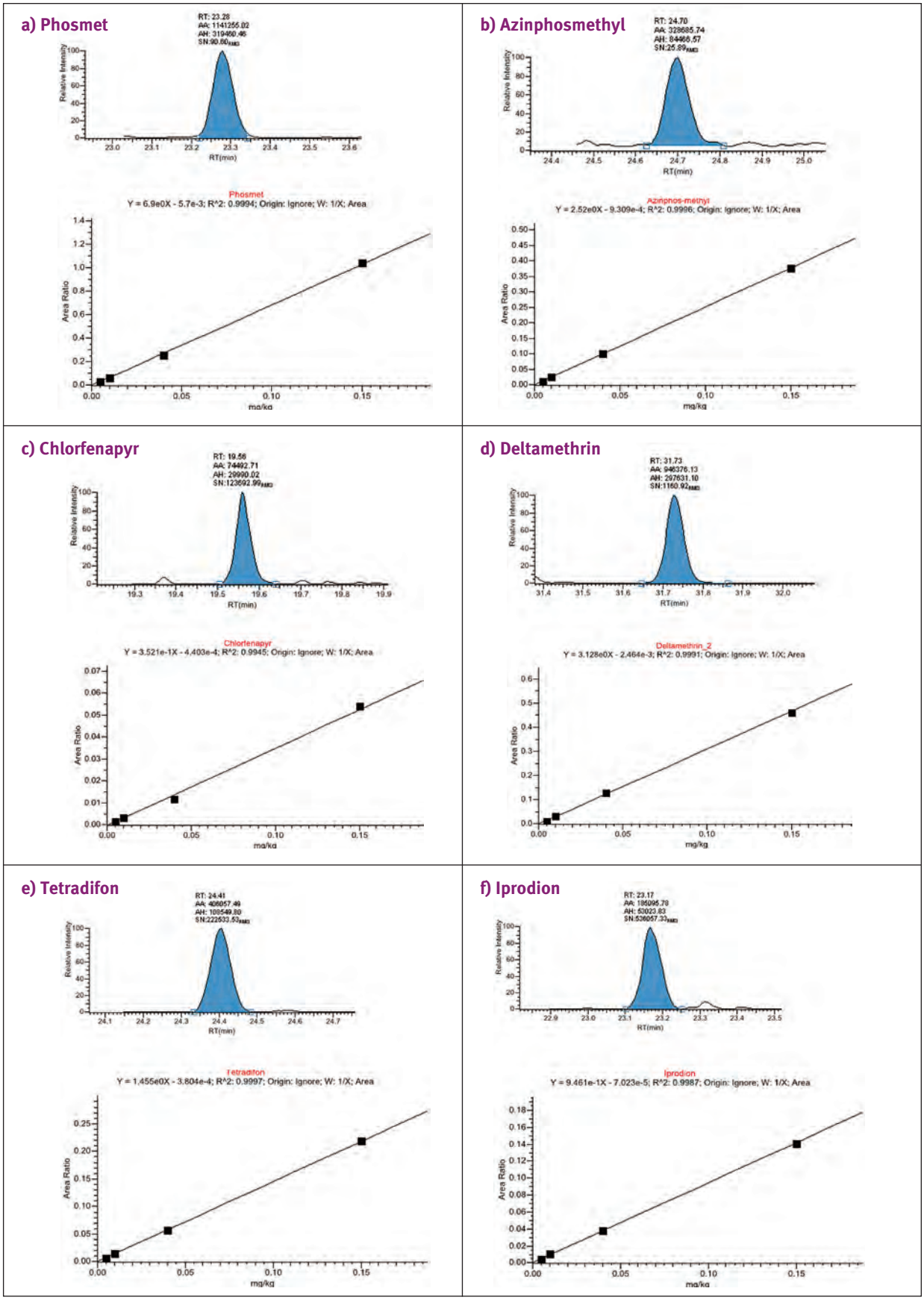


Figure 3: Calibration curves and peaks at 1 ppb level with 2 µL injection

As an additional test, the ion ratio at different levels has been monitored and the deviation of the transitions has been calculated.

Compound	Ion Ratio Deviation RSD in % (n=5)
Phosmet	0.79
Azinphosmethyl	3.65
Chlorfenapyr	15.08
Deltamethrin	0.88
Iprodion	5.34
Alfa Endosulfan	3.63
Methidathion	0.84
Carbaryl	3.64
Cyfluthrin	3.55
Pyrimifos	3.83

Table 3: Ion ratio deviation of some challenging pesticides in cucumber matrix at several levels of concentration

Figure 3 (a) through (f) show a 1 ppb matrix spike and calibration data obtained for select targeted pesticides in cucumber matrix.

Conclusions

- Advances in HyperQuad technology offers increased analytical performance for routine applications such as pesticide analysis.
- A true multi-compound method was developed for over 400 pesticides using timed SRM; easily transferable from a spreadsheet.
- A high level of accuracy and precision was reached during data evaluation, on several cornerstones of analysis, such as repeatability, linearity and ion ratio stability.
- Furthermore, all examples shown are the more challenging pesticides faced analytically in terms of stability, activity and response.
- This resolution technology development allows for advanced GC-MS/MS operations to be performed, such as U-SRM to further increase selectivity in complex matrices. This not only improves quantitative measurements, but it is also amenable when using a reduced sample clean-up which is typical for QuEChERS methodologies.

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3. Munari, F., Huebschmann, H.J., Eliminating High Boiling Matrix in GC and GC/MS by Using PTV Backflush Injection Technique for Increased Productivity and Reliability, Application Note 51888, Thermo Fisher Scientific, 2010.
4. Pesticides Method Reference, 2nd ed. 2011, Thermo Fisher Scientific, Austin, TX, USA, (part number 120390).

For reference on Thermo Scientific QuEChERS products, please see our catalog, Thermo Scientific HyperSep Dispersive SPE Products (part number BRGSCQUECHERS 1109).

AN52279_E 08/12M

Multi-Residue Pesticide Analysis in Herbal Products Using Accelerated Solvent Extraction with a Triple Quadrupole GC-MS/MS System

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Application Note 52291

Key Words
Pesticides, Tea, Herbal products, ASE, SRM, MRM, Multi-residue analysis, TSQ 8000 GC-MS/MS

Introduction

The residue analysis of pesticides has developed in recent years into a comprehensive methodology for the detection of many hundreds of potential contaminating compounds. A multi-residue method for herbal products and teas is faced with additional challenges from the worldwide origin of the products and the complex matrix of the dried materials. In the due quality control of raw materials, the unknown or undeclared local plant protection treatments must be taken into account with a wide variety of potential pesticide contaminations.

Dried leaves, fruits or seeds and other herbal products of medical use deliver highly complex extracts from the sample preparation due to the rich content of active ingredients, essential oils and the typical high boiling natural polymer compounds from broken cells, leaves or fruit skins. A thorough clean up of the extracted sample can lead to losses of critical analytes of interest. A complete characterization of pesticide, and other residue, contamination is done by both LC and GC-MS/MS to cover the complete range of functional groups.

This application report describes the methodology used for the multi-residue pesticide analysis of herbal products using accelerated solvent extraction (ASE) and gel permeation chromatography (GPC) sample preparation with detection and quantitation by the Thermo Scientific TSQ 8000 GC-MS/MS system.

A routine screening method for more than 200 pesticide compounds was applied to a wide variety of different sample types, ranging from regular black tea or sage leaves, to seeds like fennel and herbs of medical and fragrance use like thyme and chamomile. The data processing and reporting was achieved by using the Thermo Scientific TraceFinder quantitation software suite.

The sensitivity requirement for this analysis was determined by the regulatory background. The analysis of pesticide residues in tea and herbal products follows the regulations of the European Directorate General for Health and Consumer Affairs (SANCO) for “Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed” [1]. The sensitivity requirements for these products as referenced in the Codex Alimentarius [2] result in maximum residue levels of 0.01 mg/kg for most of the pesticide compounds.

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Sample Preparation

Herbal and tea samples were extracted with an accelerated solvent extraction method using the Thermo Scientific Dionex ASE 350 Accelerated Solvent Extractor. The ASE method used is described in an official pesticide standard method [3]. The collected extracts were concentrated using a rotary evaporator (Rotavap) and further cleaned up via gel permeation chromatography (GPC). The GPC step used a polystyrene gel (Bio-Beads® S-X3) with an ethylacetate/cyclohexane mobile phase. After additional concentration by the Rotavap, the extracts were ready for GC injection using ethylacetate as the main solvent.

Method Setup

The analytical method comprised sample handling and injection using the Thermo Scientific TriPlus RSH liquid autosampler, TRACE GC 1310 gas chromatograph equipped with an instant connect, temperature programmable PTV injection system, and the TSQ™ 8000 triple quadrupole GC-MS/MS detection system. The MRM detection method was taken from a routinely employed Thermo Scientific TSQ Quantum XLS GC-MS/MS method without any further optimization on the TSQ 8000 GC-MS/MS system [4]. The TSQ 8000 system automatically optimized acquisition windows and optimized instrument duty cycle using timed-SRM (t-SRM) for maximum sensitivity. This enabled the avoidance of lengthy manual set-ups usually required when adopting new instrumentation (Figure 1).

ASE™ 350 Accelerated Solvent Extraction

Sample weight	10 g
Extraction solvent	Ethylacetate/cyclo-Hexane 1:1, same as GPC solvent
Temperature	120 °C
Pressure	100 bar
Extraction time	5 min, 1 cycle
Flushing with solvent	60% of cell volume
Flushing with nitrogen	100 s

TriPlus™ RSH Autosampler

Syringe	10 µL
Injection volume	1 µL
Injection type	Fast liquid band injection, 100 ms injection time
Washing cycles	3 x 10 µL, solvent ethylacetate

TRACE™ 1310 Gas Chromatograph

Injector PTV	Splitless mode
Base temperature	50 °C
Transfer	10 °C/s to 250 °C, until end of run
Flow	Constant flow, 1.2 mL/min, helium
Analytical column	40 m, ID 0.18 mm, 0.18 µm film, 5%-phenyl phase (5MS type)
Pre-column	5 m, ID 0.18 mm, empty deactivated, no backflush
Column oven	Temperature programmed
Start	70 °C, for 1.50 min
Ramp 1	15 °C/min to 190 °C
Ramp 2	7 °C/min to 290 °C, 12 min
Transfer line	250 °C

TSQ 8000 Mass Spectrometer

Ion source temperature	220 °C
MRM Detection	Timed SRM mode (see Appendix)

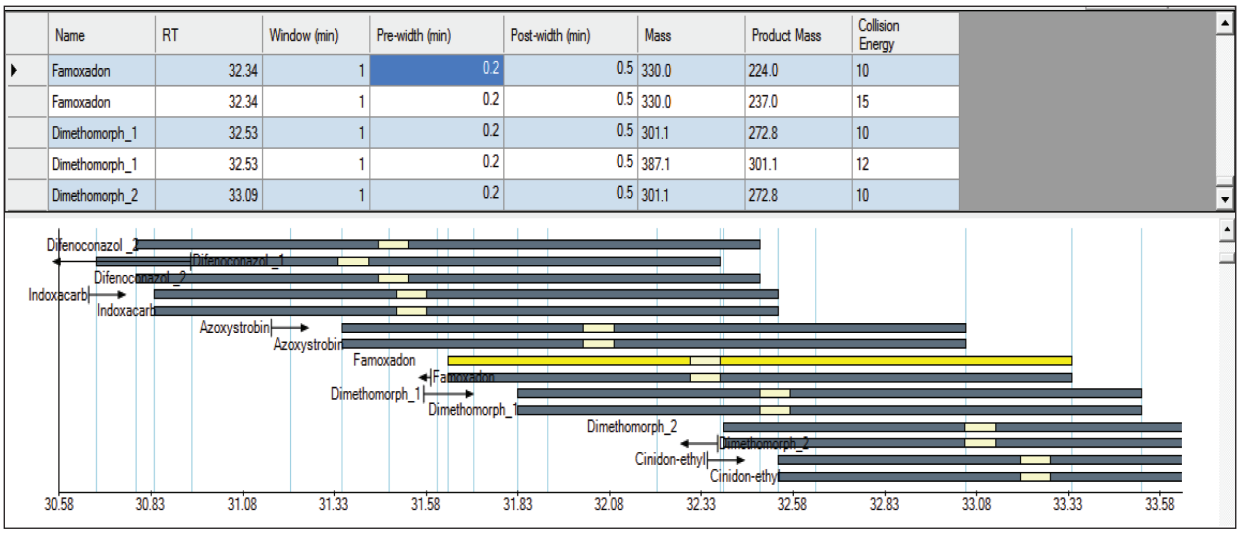

Figure 1. Screenshot of a section of the analytical run showing the "acquisition map" automatically created by the TSQ 8000 system using t-SRM. This mode ensures the instrument only monitors for compounds when they elute to optimize sensitivity.

Calibration and Linearity

The quantitative calibration and linearity check for the method was performed by using six calibration points in the range of 0.004 µg/mL to 1.0 µg/mL. This range represents an analyte concentration of 0.01 to 2.5 mg/kg in the samples (10 – 2500 ppb).

For setting up the calibration solutions, a stock solution containing target pesticide compounds in herbal products was used. The calibration solution was prepared in a standard matrix with a matrix load equivalent to the typical herbal extracts used. The standard matrix blank consisted of lemon peel extracted using the standard procedure. The pesticide blank level was tested before applying as a blank standard matrix. Standard solutions were prepared containing lemon peel extract dissolved 1:1 with ethyl acetate. The correlation coefficients, R^2, achieved during method calibration exceeded 0.99 for all compounds (Figure 2).

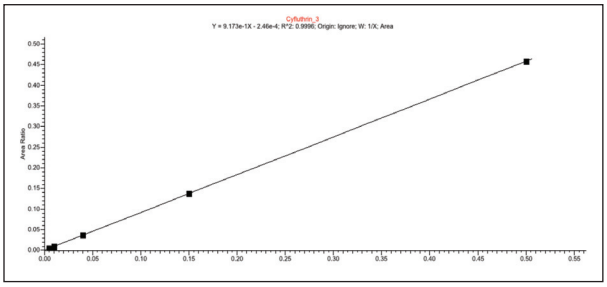

Figure 2. Calibration curve for Cyfluthrin, $R^2 = 0.9996$

Results and Discussion

Sensitivity (LOD)

Using the standard pool of pesticides, the method detection limits in the standard lemon peel were estimated. Using the 4 ppb (pg/µL) matrix standard level, S/N values were used to estimate the limits of detection (LOD). The S/N values in matrix are given in Table 1 for a selection of critical compounds taken at retention times that are affected most from the eluting matrix. Although the compounds are eluting in heavily impacted matrix regions of the chromatogram, the high selectivity of the TSQ 8000 GC-MS/MS for the target pesticides at low level against an intense matrix load is demonstrated in Figure 3 and Figure 4.

Table 1. Detection limit S/N for selected pesticide compounds in matrix

Pesticide	RT [min]	S/N @ 4 ppb
Terbacil	13:83	24
Alachlor	14:78	12
Tolylfluanid	16:75	44
Pyridaben	24:17	83

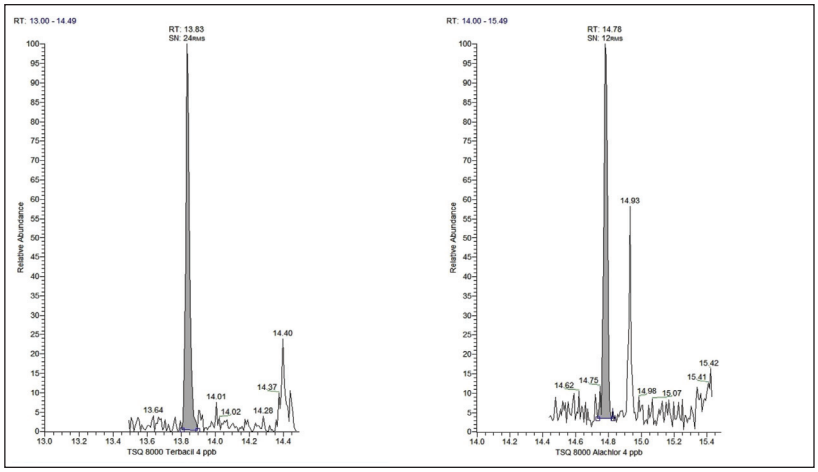

Figure 3. SRM peaks at 4 ppb from Terbacil (left, 161.1 › 88.0, CE 15 V) and Alachlor (right, 188.1 › 130.1, CE 25 V). SRM transitions were taken from the Pesticide Method Reference, 2nd ed. 2011. [4]

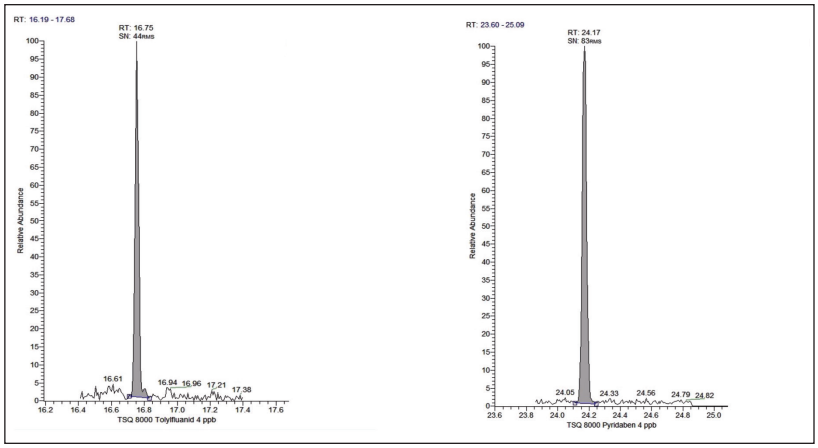

Figure 4. SRM peaks at 4 ppb from Tolylfluanid (left, 238.1 › 137.1, CE 15 V) and Pyridaben (right, 309.1 › 147.1, CE 15 V). SRM transitions were taken from the Pesticide Method Reference, 2nd ed. 2011. [4]

Robustness and Maintenance

Routine preventative maintenance on the GC was performed using routine standard operating procedures. The calibration chromatograms seen in Figures 3 and 4 have been acquired after a persistent matrix load to the system through routine analysis of more than 500 matrix samples.

This level of robustness meant that even with persistent and very high matrix load, it was not necessary to clean the removable ion source short term.

The innovative instant connect modularity of the injectors and detectors of the TRACE 1310 GC, used here as the front-end to the mass spectrometer, allows the user quick accessibility to any injector part for rapid cleaning. Furthermore the unique ability to replace the entire injector module within minutes represents an excellent way of postponing routine maintenance to when the laboratory schedule allows while keeping the GC-MS/MS system operational.

Analytical Precision

Within a routine series of 50 commercial samples, the quality control samples were measured with replicate injections. The results for a range of compounds is given in Table 2. The relative effects on known problematic pesticide compounds can be seen, while coefficients of variation (CV%) for unaffected compounds all stay well below 10% even within this long series of matrix injections.

Table 2. Coefficients of variation for lemon peel matrix spiked QC samples for a set of 60 pesticides under investigation (avg. 7.4%, 24 injections)

Diflubenzofuron	10.0%	Penconazol	7.5%	Diniconazol	2.9%
Biphenyl-d10	7.5%	Allethrin	8.4%	Aclonifen	9.0%
Biphenly	9.5%	Pyrifenox	5.5%	Trifloxystrobin	6.0%
o-Phenylphenol	8.2%	Procymidon	5.7%	Propiconazol	3.1%
Fenobucarb	6.0%	Triadimenol	11.5%	Propargit	6.0%
Diphenylamin	5.7%	Picoxystrobin	7.0%	Tebuconazol	4.3%
Terbutylazin	4.4%	Flutriafol	6.3%	Nitralin	9.2%
Propyzamid	3.1%	Hexaconazol	9.2%	Piperonyl butoxid	8.3%
Terbazil	5.8%	Isoprothiolan	9.7%	Brompropylat	5.8%
Fipronil-desulfinyl	6.9%	Uniconazol	7.0%	Fenoxycarb	9.1%
Alachlor	6.7%	Kresoxim-methyl	9.9%	Etoxazol	8.8%
Prometryn	8.3%	Myclobutanil	9.2%	Fenazaquin	3.3%
Ethofumesat	7.4%	Flusilazol	4.4%	Metconazol	5.3%
Bromacil	8.3%	Cinerin 1	8.1%	Pyriproxyfen	8.5%
Chlorpyrifos	6.9%	Buprofezin	7.4%	Fenamirol	8.5%
Tetraconazol	6.2%	Diclobutrazol	2.6%	Fluquinconazol	4.9%
Triadimefon	11.7%	Cyproconazol	2.6%	Pyridaben	5.2%
Dicapton	10.7%	Chlorbenzilat	3.3%	Etofenprox	10.2%
Butralin	6.6%	Etoconazol	4.4%	Silafluofen	10.2%
Fipronil	5.5%	Iprodion	11.1%	Indoxacarb	8.5%

Results from Real Life Samples

The above method was used for the analysis of a wide variety of herbs, teas and dried fruit known as one of the most challenging analytical task for controlling the pesticide maximum residue levels due to the heavy matrix impact. Table 3 gives a representative overview of positive results from different samples with the indication of the pesticide compound and concentration found. All compounds were detected by using at least two SRM traces and were subsequently confirmed by checking the calibrated ion ratios. The concentration ranges covered were from close to the MRL level of 10 mg/kg to high levels of up to 50 times above the regulated maximum. Figure 5 provides an example of confirmed residue detection in a thyme sample.

Table 3. Positive results above MRL level found in samples of various matrices

Sample Matrix	Pesticide Residues Found	Concentration (mg/kg)
Dried Herbs	o-Phenylphenol	0.017
Dried Herbs	Tebuconazol	0.023
Dried Fruit	Diflubenzuron	0.049
Dried Fruit	Myclobutanil	0.023
Dried Fruit	Propargit	0.479
Dried Fruit	Tebuconazol	0.081
Dried Fruit	Difenconazol	0.013
Dried Herbs	Picoxystrobin	0.228
Dried Herbs	Picoxystrobin	0.233
Dried Herbs	o-Phenylphenol	0.011
Herbal Tea	o-Phenylphenol	0.014
Herbal Tea	o-Phenylphenol	0.011
Herbal Tea	Terbutylazin	0.016

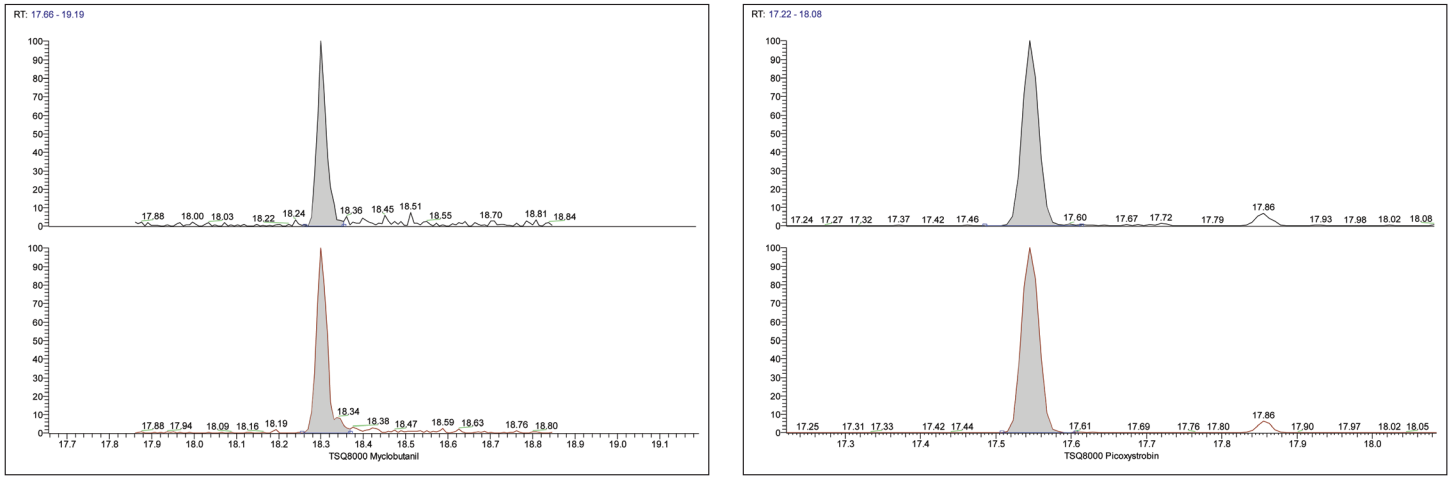

Figure 5. Positive results for Myclobutanil in green apple (0.023 mg/kg, left) and Picoxystrobin in thyme (0.228 mg/kg, right), both detected on two SRM traces

Data Analysis and Reporting

The data processing was performed using TraceFinder™ quantitation software. TraceFinder software contains a compound data store containing a large number of pesticide compound entries from which required compounds for the method had been selected. For each pesticide, the necessary parameters for MRM acquisition and compound identification, such as SRM transition, retention time, and ion ratios, as well as quantitation details like quantitation mass and recovery requirement, are stored.

The analytical sequence setup, data acquisition and result processing was done from one software platform integrating the complete analytical process. In Figure 6, the analytical sequence is shown in the upper part of the screen, with the compounds included in the method to the right. The actual chromatograms for the selected pesticide compounds are displayed in the bottom part for review by the operator.

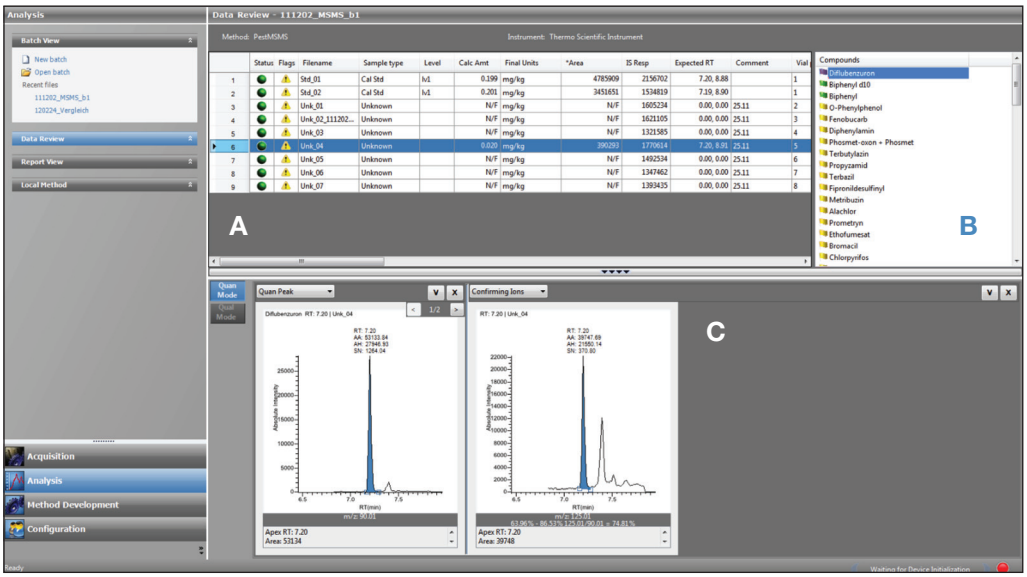

Figure 6. TraceFinder software analysis view:

A. Acquisition sequence table for calibration, QC and sample runs

B. Compound list with status flags

C. Compound chromatogram windows with integrated quantitation and confirmation peaks

Expanded Productivity

The total cycle time of the analytical runs was 30 minutes, which allowed the throughput of two samples per hour and resulted in a load of up to 48 samples, including QC checks during the day for the control of more than 200 pesticide compounds in each run.

This expanded productivity was a combined result of the TSQ 8000 triple quadrupole GC-MS/MS system with its enhanced analyte selectivity in matrix samples, the high method and system robustness, and the advanced data processing using TraceFinder software. Pesticide peaks were typically baseline-separated with a high signal-to-noise ratio allowing for an accurate automated area integration with significantly reduced manual control required. A number of quality control parameters within TraceFinder software immediately provided visible flagging for compounds that may need manual attention. Automatic ion ratio checks provided a fast and solid confirmation in the case of positive findings. The high processing speed of TraceFinder software provided for multi-residue analysis and quick and comprehensive reporting for each sample.

Conclusion

The TSQ 8000 GC-MS/MS delivered high sensitivity and matrix selectivity for routine pesticide analysis even in difficult matrix samples. The data acquisition using the unique timed-SRM allowed for the detection of a virtually unlimited number of pesticide compounds in one run without sacrificing the high sensitivity for individual compounds. Quantitative calibrations were performed in a standard matrix and showed excellent linearity and precision over the relevant concentration range to control the regulated MRL levels.

The high matrix selectivity of the TSQ 8000 system allowed for reduced sample preparation, providing high recoveries for a wide range of chemically diverse pesticide compounds. The very high matrix selectivity delivered low chemical matrix background with well-defined pesticide peaks that were safe and easy to integrate, thus eliminating the need for time-consuming manual baseline corrections.

Positive pesticide compound signals were confirmed by TraceFinder software checking the calibrated ion ration of the two monitored SRM transitions.

The TSQ 8000 GC-MS/MS system is well prepared for routine analysis and provides high robustness of the chromatographic system and ion source, thus reducing the need for frequent maintenance and avoiding system downtime for high sample throughput and productivity. The system is easy to use, durable, and robust even with the most challenging sample types and is fully automated in sampling capabilities to found and not-found report generation.

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4. Pesticide Method Reference, 2nd Edition, 2011 Thermo Fisher Scientific, p/n 120390.

Appendix: List of pesticides with MRM transitions used (from [4])

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Difluorobenzamid Degradation (Isocyanat)	6.93	152.93	90.01	20
Difluorobenzamid Degradation (Isocyanat)	6.93	152.93	125.01	20
Carbofuran 1	8.80	149.06	121.05	10
Carbofuran 1	8.80	164.08	149.07	10
Difluorobenzamid Degradation	8.62	141.00	63.11	25
Difluorobenzamid Degradation	8.62	141.00	113.09	15
Biphenyl-d10_ISTD	9.24	160.00	160.16	10
Biphenyl	9.28	154.08	153.08	15
Biphenyl	9.28	153.08	152.08	15
Carbofuran-3-hydroxy 1	10.43	137.05	81.01	18
Carbofuran-3-hydroxy 1	10.43	180.05	137.01	15
Tetrahydrophthalimid	10.84	151.04	79.01	25
Tetrahydrophthalimid	10.84	151.04	122.09	10
O-Phenylphenol	11.00	170.07	141.06	20
O-Phenylphenol	11.00	170.07	115.05	20
Molinate	11.10	187.10	126.07	10
Molinate	11.10	126.07	98.05	5
Chlorfenprop methyl	11.59	196.00	165.00	10
Chlorfenprop methyl	11.59	165.00	137.00	10
Fenobucarb	11.20	121.07	77.05	15
Fenobucarb	11.20	150.09	121.07	10
Propachlor	11.76	176.06	120.04	10
Propachlor	11.76	120.04	92.03	10
Propachlor	11.76	169.06	120.04	10
Propachlor	11.76	196.07	120.04	10
Cycloate	11.98	154.10	83.05	10
Cycloate	11.98	215.13	154.10	5
Diphenylamin	11.49	169.01	168.09	20
Diphenylamin	11.49	169.01	167.09	20
Chloropropham	12.26	213.06	127.03	15
Chloropropham	12.26	213.06	171.04	10
Phosmet-oxon	12.09	160.00	132.96	15
Phosmet-oxon	12.09	104.00	75.88	10
Phosmet-oxon	12.09	160.00	76.96	20
Prometon	13.10	225.16	183.13	10
Prometon	13.10	225.16	210.15	10
Carbofuran 2	13.13	149.06	121.05	10
Carbofuran 2	13.13	164.08	149.07	10
Profluralin	13.22	318.10	199.06	15
Profluralin	13.22	330.23	252.45	25
Swep	13.46	187.05	123.95	18
Swep	13.46	219.11	174.02	15
Trietazine	13.48	229.14	200.14	15
Trietazine	13.48	214.14	186.10	15
Dimethipin	13.53	117.98	57.97	10
Dimethipin	13.53	210.10	76.02	10
Terbutylazin	12.97	214.10	132.06	10
Terbutylazin	12.97	214.10	104.05	10
Propyzamid	13.04	173.01	145.01	15
Propyzamid	13.04	173.01	109.01	18
Propyzamid	13.04	175.02	147.01	15
Propyzamid	13.04	254.02	226.02	15
Isocarbamide	13.67	142.03	70.01	15
Isocarbamide	13.67	142.03	113.01	10
Dinoseb	13.92	211.13	116.99	15
Dinoseb	13.92	211.13	163.11	10
Terbazil	13.42	161.05	88.03	15
Terbazil	13.42	160.05	76.02	15
Bromocylen	14.37	358.79	242.85	15
Bromocylen	14.37	356.93	241.24	15
Dimethenamid	14.60	230.06	154.04	10
Dimethenamid	14.60	232.06	154.04	10
Dimethachlor	14.61	197.08	148.06	10
Dimethachlor	14.61	199.08	148.06	10
Acetochlor	14.65	174.11	146.15	15
Acetochlor	14.65	223.19	147.17	10
Desmetryn	14.68	213.11	171.08	10
Desmetryn	14.68	213.11	198.10	10
Flurprimidol	14.77	269.12	106.98	20
Flurprimidol	14.77	270.18	107.04	20
Alachlor	14.26	188.10	160.07	10
Alachlor	14.26	188.10	130.12	25
Alachlor	14.26	237.14	160.15	10
Metribuzin	14.14	198.08	82.03	20
Metribuzin	14.14	198.08	89.04	16
Propanil	15.00	217.01	161.00	10
Propanil	15.00	219.01	163.00	10
Fipronildesulfinyl	14.15	333.00	231.20	20
Fipronildesulfinyl	14.15	333.00	281.30	20
Carbofuran-3-hydroxy 2	15.02	137.05	81.01	18
Carbofuran-3-hydroxy 2	15.02	180.05	137.01	15
Prometryn	14.49	241.14	184.10	15
Prometryn	14.49	226.13	184.10	12
Tridiphan	15.18	186.94	158.94	15
Tridiphan	15.18	219.09	184.09	20
Ethofumesat	14.80	206.82	160.86	10
Ethofumesat	14.80	285.75	206.82	12
Pentanochlor	15.73	141.05	106.05	15
Pentanochlor	15.73	239.05	141.05	15
Chlorpyrifos	15.78	257.97	165.98	20
Chlorpyrifos	15.78	314.05	258.18	15
Bromacil	15.03	205.01	188.01	15
Bromacil	15.03	207.01	190.01	15

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Anthrachinon	15.44	207.97	151.99	20
Anthrachinon	15.44	180.04	152.05	15
Anthrachinon	15.44	207.97	180.10	10
Nithrothal isopropyl	16.09	236.08	194.07	10
Nithrothal isopropyl	16.09	236.08	148.05	20
Triadimefon	15.41	208.07	181.06	10
Triadimefon	15.41	210.07	183.06	10
Tiocarbazil	16.15	156.08	100.05	8
Tiocarbazil	16.15	279.10	156.07	6
Tetraconazol	15.39	336.02	218.01	20
Tetraconazol	15.39	338.02	220.01	20
Butralin	15.54	266.14	220.11	15
Butralin	15.54	266.14	190.10	15
Dicapthon	15.44	262.00	262.00	9
Dicapthon	15.44	262.00	216.00	13
Crufomat	16.30	256.20	226.15	25
Crufomat	16.30	276.20	182.09	10
Allethrin	16.17	123.07	80.98	10
Allethrin	16.17	136.04	92.98	10
Dinobuton	16.89	163.06	116.04	15
Dinobuton	16.89	211.07	117.04	18
Penconazol	16.89	248.06	157.04	25
Penconazol	16.89	248.06	192.04	15
Pyrifenox 1	16.17	262.03	192.02	20
Pyrifenox 1	16.17	262.03	200.02	20
Pyrifenox 2	16.81	262.03	192.02	20
Pyrifenox 2	16.81	262.03	200.02	20
Tolylfluanid	16.92	238.09	137.05	15
Tolylfluanid	16.92	240.09	137.05	15
Fipronil	17.01	368.95	214.97	30
Fipronil	17.01	366.95	254.96	25
Triflumizol	17.20	206.05	179.04	15
Triflumizol	17.20	179.04	144.04	15
Procymidon	17.22	283.05	95.93	10
Procymidon	17.22	285.05	95.97	10
Procymidon	17.22	285.05	257.30	10
Triadimenol 1	16.45	168.11	69.99	15
Triadimenol 1	16.45	128.05	100.04	10
Triadimenol 2	16.64	168.11	69.99	15
Triadimenol 2	16.64	128.05	100.04	10
Butachlor	17.54	237.13	160.09	10
Butachlor	17.54	176.09	146.08	10
Chlorbenside	17.57	124.97	88.98	20
Chlorbenside	17.57	124.97	63.02	30
Fenothiocarb	17.68	160.07	72.01	15
Fenothiocarb	17.68	160.07	106.00	10
Picoxystrobin	17.69	335.09	303.09	10
Picoxystrobin	17.69	303.09	157.04	20
Paclobutrazole	17.75	236.10	125.06	15

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Paclobutrazole	17.75	238.11	127.06	15
Chinomethionat	17.78	206.06	147.98	15
Chinomethionat	17.78	234.08	206.06	10
Napropamid	18.07	271.16	128.07	5
Napropamid	18.07	128.07	72.04	10
Flutriafol	18.11	219.07	123.04	15
Flutriafol	18.11	123.04	75.03	15
Flurodifen	18.14	190.02	126.01	10
Flurodifen	18.14	190.02	146.01	5
Bisphenol A	18.17	213.14	119.06	15
Bisphenol A	18.17	213.14	164.99	20
Bisphenol A	18.17	228.15	213.07	10
Chlorfenson_ISTD	18.20	302.00	110.90	20
Hexaconazol	18.22	214.08	159.07	20
Hexaconazol	18.22	214.08	151.98	25
Imazalil	18.24	172.96	144.96	15
Imazalil	18.24	172.96	108.95	25
Isoprothiolan	18.24	203.99	117.95	7
Isoprothiolan	18.24	203.99	84.90	25
Isoprothiolan	18.24	290.06	118.03	15
Flamprop-methyl	18.39	230.05	170.04	10
Flamprop-methyl	18.39	276.06	105.02	10
Kresoximmethyl	18.48	206.10	131.09	15
Kresoximmethyl	18.48	206.10	116.01	10
Buprofezin	18.51	175.08	116.96	20
Buprofezin	18.51	175.08	131.99	15
Buprofezin	18.51	249.16	105.93	20
Buprofezin	18.51	249.16	193.20	10
Uniconazol	18.57	234.12	136.99	15
Uniconazol	18.57	234.12	101.95	25
Uniconazol	18.57	234.12	165.08	10
Cinerin 1	18.60	123.08	95.06	10
Cinerin 1	18.60	123.08	81.05	10
Cinerin 1	18.60	150.10	108.09	10
Flusilazol	18.60	233.16	165.13	25
Flusilazol	18.60	233.16	152.06	20
Myclobutanil	18.65	179.00	125.00	15
Myclobutanil	18.65	179.00	89.95	25
Methoprotryne	18.66	256.14	212.11	15
Methoprotryne	18.66	256.14	200.11	15
Diclobutrazol	18.75	270.07	159.04	15
Diclobutrazol	18.75	272.08	161.04	15
Azaconazole	18.78	217.02	173.01	15
Azaconazole	18.78	219.02	175.01	15
Perthane	18.95	223.15	179.10	18
Perthane	18.95	223.15	167.06	18
Cyproconazol	19.14	222.09	125.05	20
Cyproconazol	19.14	224.09	127.05	20
Flamprop-isopropyl	19.14	276.08	105.03	15

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Flamprop-isopropyl	19.14	278.17	104.99	20
Chloropropylat	19.16	251.02	139.01	20
Chloropropylat	19.16	251.02	111.01	20
Ancymidol	19.18	228.15	121.02	15
Ancymidol	19.18	215.15	107.02	15
Chlorbenzilat	19.22	251.02	139.01	20
Chlorbenzilat	19.22	251.02	111.01	20
Cyprofuram	19.36	211.12	132.02	10
Cyprofuram	19.36	211.12	166.05	10
Etaconazol 1	19.38	245.04	173.03	15
Etaconazol 1	19.38	245.04	191.03	10
Etaconazol 2	19.38	245.04	173.03	15
Etaconazol 2	19.38	245.04	191.03	10
Diniconazol	19.47	268.06	232.05	15
Diniconazol	19.47	270.06	234.05	15
Jasmolin 1	19.58	123.08	81.05	10
Jasmolin 1	19.58	123.08	95.06	10
Jasmolin 1	19.58	164.16	109.15	10
Aclonifen	19.70	212.02	182.02	10
Aclonifen	19.70	264.03	194.02	15
Tetrasul	19.85	251.92	216.93	20
Tetrasul	19.85	253.92	218.93	20
Carfentrazone ethyl	19.95	340.03	312.03	10
Carfentrazone ethyl	19.95	312.15	150.99	20
Benodanil	19.99	322.98	230.99	15
Benodanil	19.99	322.98	195.99	5
Trifloxystrobin	20.02	222.13	162.14	10
Trifloxystrobin	20.02	115.99	88.95	15
Trifloxystrobin	20.02	222.13	130.02	15
Chlordecone	20.06	271.91	237.16	15
Chlordecone	20.06	273.91	239.15	20
Famophos (Famphur)	20.16	218.07	108.94	15
Famophos (Famphur)	20.16	218.07	126.95	20
Iprodion Degradation	18.63	186.87	123.99	20
Iprodion Degradation	18.63	186.87	159.02	15
Iprodion Degradation	18.63	243.94	187.02	10
Iprodion	20.57	314.06	245.25	15
Iprodion	20.57	186.99	123.87	20
Iprodion	20.57	316.00	247.35	15
Iprodion	20.57	316.00	273.11	10
Propiconazol 1	19.38	259.02	173.02	20
Propiconazol 1	19.38	172.94	144.91	15
Propiconazol 2	19.54	259.02	173.02	20
Propiconazol 2	19.54	172.94	144.91	15
Pyraflufen-ethyl	20.30	412.02	349.02	15
Pyraflufen-ethyl	20.30	349.02	307.02	15
Clodinafop-propargyl	20.36	349.05	266.04	15
Clodinafop-propargyl	20.36	349.05	238.04	15
Lenacil	20.70	153.05	136.06	15

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Lenacil	20.70	153.05	135.15	15
Diclofop methyl	20.77	253.02	162.01	15
Diclofop methyl	20.77	340.04	253.02	15
Propargit	20.79	173.08	135.04	15
Propargit	20.79	173.08	106.93	20
Propargit	20.79	350.21	173.10	15
Diflufenican	20.83	394.07	266.05	10
Diflufenican	20.83	266.05	246.05	10
Piperonylbutoxid	20.87	176.11	131.08	15
Piperonylbutoxid	20.87	176.11	103.06	10
Piperonylbutoxid	20.87	176.11	145.09	15
Tebuconazol	20.97	250.12	125.06	20
Tebuconazol	20.97	252.12	127.06	20
Nitralin	21.09	316.02	274.15	10
Nitralin	21.09	273.99	216.07	10
Benzoylpropethyl	21.22	292.05	105.02	15
Benzoylpropethyl	21.22	172.03	145.02	14
Captafol	21.22	311.06	78.94	20
Captafol	21.22	311.06	276.21	10
Epoxyconazol	21.29	192.04	138.03	10
Epoxyconazol	21.29	192.04	111.02	10
Bromuconazol 1	21.73	294.96	174.98	15
Bromuconazol 1	21.73	292.96	172.98	15
Brompropylat	21.76	340.93	183.05	20
Brompropylat	21.76	340.93	185.04	20
Etoxazol	21.83	300.14	270.38	20
Etoxazol	21.83	330.17	300.44	25
Fenoxycarb	21.85	186.08	109.05	15
Fenoxycarb	21.85	255.11	186.08	10
Phosmet	20.79	160.00	133.00	15
Phosmet	20.78	160.00	104.00	20
Phosmet	20.78	316.99	160.00	5
Fenpiclonil	21.94	235.99	200.99	15
Fenpiclonil	21.94	237.99	200.99	15
Fenazaquin	22.22	160.09	145.08	10
Fenazaquin	22.22	145.05	116.99	15
Fenazaquin	22.22	160.09	117.08	20
Phenothrin 1	22.27	183.10	153.08	18
Phenothrin 1	22.27	183.10	165.09	10
Phenothrin 2	22.42	183.10	153.08	18
Phenothrin 2	22.42	183.10	165.09	10
Bromuconazol 2	22.35	294.97	174.97	15
Bromuconazol 2	22.35	292.97	172.97	15
Metconazol	22.41	125.00	88.93	20
Metconazol	22.41	250.20	124.88	25
Triticonazole	22.80	235.10	217.09	10
Triticonazole	22.80	235.10	182.07	10
Pyriproxyfen	22.82	226.15	186.22	15
Pyriproxyfen	22.82	136.00	95.95	15

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Azinphosmethyl	22.95	160.00	132.00	10
Azinphosmethyl	22.95	160.00	104.64	10
Pyriproxyfen	23.06	136.00	77.92	20
Fenamirol	23.55	251.02	139.01	15
Fenamirol	23.55	330.03	139.01	10
Pyridaben	24.50	364.14	309.12	5
Pyridaben	24.50	309.12	147.06	15
Fluquinconazol	24.59	340.01	298.01	22
Fluquinconazol	24.59	342.01	300.01	22
Etofenprox	26.05	163.09	107.06	16
Etofenprox	26.05	163.09	135.07	10
Etofenprox	26.05	376.14	135.02	30
Etofenprox	26.05	376.14	163.09	10
Silafluofen	26.25	179.00	151.00	7
Silafluofen	26.25	286.13	258.12	15
Difenconazol 1	26.91	323.05	265.04	15
Difenconazol 1	26.91	325.05	267.04	20
Difenconazol 2	27.05	323.05	265.04	15
Difenconazol 2	27.05	325.05	267.04	20
Indoxacarb	28.55	264.02	176.14	10
Indoxacarb	28.55	264.02	148.03	20
Indoxacarb	28.55	321.05	289.34	10

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Comparing LC and GC Triple Quadrupole MS for the Screening of 500 Pesticides in Matrix

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Overview

Purpose: The goal of this project is to compare the screening of more than 500 pesticides in matrix by LC and GC triple quadrupole, and determine the value of a comprehensive LC and GC screening approach.

Methods: The methodology included the vegetable extraction by QuEChERS followed by GC-MS/MS and LC-MS/MS analysis of over 500 pesticides in matrix.

Results: The majority of compounds could be detected to levels acceptable by EU standards by either GC/MS or LC/MS. All but eight pesticides could be determined to acceptable levels by the combined GC/LC methodology.

Introduction

Modern pesticide analysis is extremely challenging due to the diversity of compounds required to be reported, especially in the area of food safety control. Furthermore, the pressure to report large numbers of pesticides quickly makes it attractive to use large single injection methods. Triple quadrupole mass spectrometry has emerged as a primary technique for screening large target lists of pesticides due to its high sensitivity and selectivity against matrix. However, because of the chemical diversity of pesticides, LC or GC introduction alone may not be ideal, or even sufficient for a comprehensive analysis. Presented is a comparison of both LC and GC sample introduction techniques coupled to triple quadrupole mass spectrometer for the screening of more than 500 pesticides at ppb levels.

Methods

Sample Preparation

Pesticide standards were obtained from the U.S. Food and Drug Administration (FDA). In order to determine detection limits of such a wide range of pesticides, standards were prepared at multiple levels, enabling the selection of an appropriate level to determine the detection limit of each compound.

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Vegetable matrices were prepared for analysis by using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, which is a sample preparation procedure used to extract pesticides from food[1]. The QuEChERS extracts were obtained from California Department of Food and Agriculture. For the QuEChERS extraction, 15 g of homogenized sample and 15 mL of acetonitrile were used.

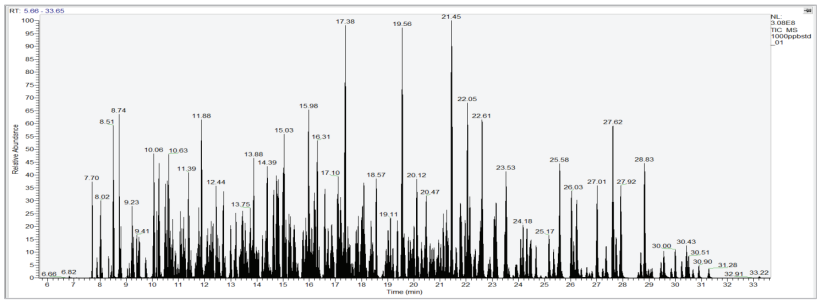
GC/MS Instrument Methodology

Gas Chromatograph Method Conditions

A method was developed for the Thermo Scientific™ TRACE™ 1310 Gas Chromatograph and Thermo Scientific™ TSQ™ 8000 Mass Spectrometer. A Programmable Temperature Vaporization (PTV) injector was used on the TRACE 1310. The ability to program a temperature ramp with this injector was utilized so that thermally labile pesticides would be transferred to the analytical column at as low a temperature possible.

Similarly, the oven on the TRACE 1310 gas chromatograph was ramped, volatilizing pesticides on the column as their boiling points were reached. A slow ramp of 5 ºC/min was employed between an oven temperature of 180 ºC and 280 ºC, which is the range in which the majority of these pesticides are volatilized, to achieve optimal separation during this most dense part of the chromatogram. Figure 1 shows the total ion chromatogram resulting from the GC/MS method, and Figure 2 lists the GC method parameters.

FIGURE 1. GC/MS Total Ion Chromatogram.



The analytical column used was a Thermo Scientific™ TraceGOLD™ TG-5SILMS, with dimensions 30 m x 0.25 mm x 0.25 µm. The liner employed was a baffled, Siltek™ deactivated inlet liner.

FIGURE 2. Gas Chromatograph Parameters.

Injection Volume	
Injection Volume (µL):	1.0
Trace 1310 GC PTV Inlet	
PTV mode:	Splitless
Inlet (°C):	75
Split flow(ml/min):	50
Splitess time (min)	1
PTV inject:	75 °C , 0.1 min to transfer step
PTV transfer:	300 °C, 2.5 °C/sec for 3 min to clean step
PTV Clean:	330 °C, 14.5 °C/sec for 20 min
Carrier Flow He (mL/min):	1.2
Oven Temperature Program	
Temperature 1 (°C):	40
Hold Time (min):	1.5
Rate (°C/min)	25
Temperature 2 (°C):	90
Hold Time (min):	1.5
Rate (°C/min)	25
Temperature 3 (°C):	180
Hold Time (min):	0
Rate (°C/min)	5
Temperature 4 (°C):	280
Hold Time (min):	0
Rate (°C/min)	10
Temperature 5 (°C):	300
Hold Time (min):	5

GC-Triple Quadrupole Method Conditions

Transitions for all pesticides were taken from the Thermo Scientific™ TSQ 8000 Pesticide Analyzer. These transitions were originally developed with the use of AutoSRM software, which provided automated SRM development with collision energies optimized to ± 1 eV. Thermo Scientific TraceFinder™ software was used for acquisition and processing of the extracted samples. Selecting the appropriate compounds from the pesticide analyzer automatically populated the SRM acquisition list in the instrument method and the compound processing parameters in the Thermo Scientific™ TraceFinder™ software processing method. One ion per compound was used for quantitation and two additional ions were used for ion ratio confirmation. Figure 3 lists additional MS parameters used.

FIGURE 3. GC-Mass Spectrometer Parameters

Mass Spec Parameters	
Transfer line (°C):	**250**
Source temperature (°C):	**300**
Mode:	**SRM**
Ionization:	**EI, 70 eV**
Collision Gas:	**Argon**
Resolution:	**Q1 normal**

LC/MS Instrument Methodology

U-HPLC Method Conditions

Chromatographic analysis was performed using the Thermo Scientific™ Accela™ 1250 UHPLC system. The autosampler was an HTC-PAL™ Autosampler (CTC Analytics, Zwingen, Switzerland). The column used was a Thermo Scientific™ Hypersil™ GOLD aQ column (100 x 2.1 mm, 1.9 μm particle size). Displayed in Figure 4 is the total ion chromatogram. The UHPLC conditions are listed in Figure 5.

FIGURE 4. LC/MS Total Ion Chromatogram

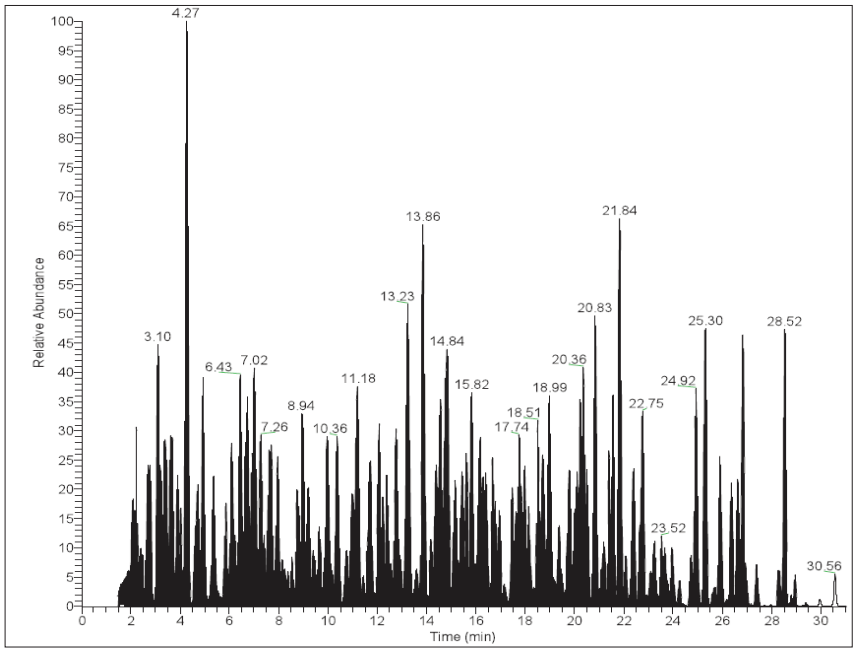


FIGURE 5. HPLC Parameters

HPLC Parameters			
Mobile Phase A:	Water with 0.1% formic acid and 4 mM ammonium formate		
Mobile Phase B:	Methanol with 0.1% formic acid and 4 mM ammonium formate		
Flow Rate:	300 µL/min		
Column Temperature:	40 °C		
Sample Injection Volume:	10 µL		
Gradient:	Gradient Time (min)	%A	%B
	0.00	98	2
	0.25	70	30
	35.00	0	100
	40.00	0	100
	40.01	98	2
	45.00	98	2

TSQ Quantum Access MAX LC-Triple Quadrupole Method Conditions

All samples were analyzed on the Thermo Scientific™ TSQ Quantum Access MAX™ triple stage quadrupole mass spectrometer with a heated electrospray ionization (HESI) source. To maximize the performance of the mass spectrometer, time-specific SRM windows were employed at the retention times of the target compounds. In addition, Quantitation-Enhanced Data-Dependent scanning, which delivers SRM-triggered MS/MS data, was used for structural confirmation. Alternating positive and negative polarity switching was utilized in the method. The MS conditions are listed in Figure 6 below.

FIGURE 6. LC-Mass Spectrometer Parameters.

Mass Spec Parameters	
Sheath Gas Flow Rate:	55 units
Aux Gas Flow Rate:	15 units
Spray Voltage:	3500 V
Capillary Temp:	280 °C
Heater Temp:	295 °C
Cycle Time:	0.2 s

Results and Discussion

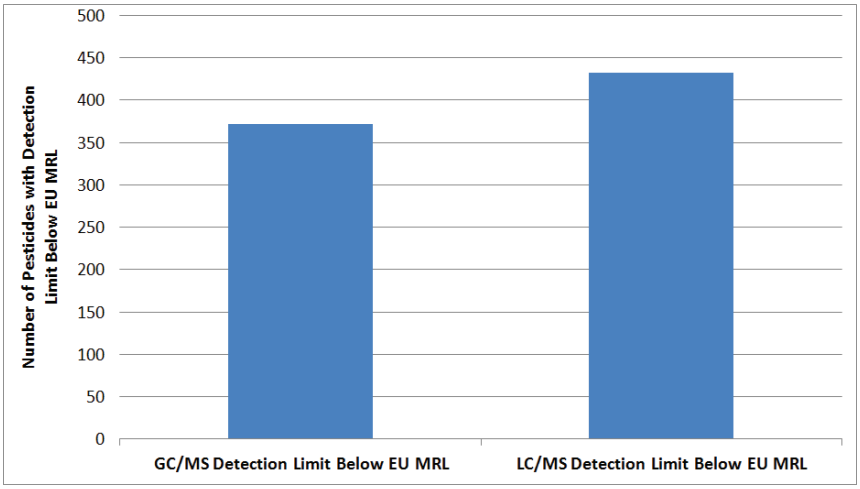
Determination of Method Detection Limit

For both GC/MS and LC/MS methods, spiked matrix samples were analyzed at several concentrations close to or below the European Union Method Reporting Limit (EU MRL). Each concentration level was injected several times and a statistical determination[2] of the method detection limit was calculated for comparison to the EU MRL for an onion matrix for each pesticide. When a required MRL was not available for the pesticide in onion, a 10 parts per billion MRL was used as stated in EU regulations.

Comparison of GC/MS to LC/MS

The majority of compounds were detected below EU MRLs by either the GC/MS or LC/MS method used (Figure 7). Out of the total 524 compounds analyzed, 372 pesticides had MDLs less than EU MRLs for the GC/MS methodology, compared with 432 pesticides with MDLs below the EU MRLs for the LC/MS methodology. Note that a10 µL injection was used in the LC/MS methodology compared with a 1 µL injection employed in the GC/MS methodology.

FIGURE 7. Number of compounds with method detection limits lower than EU MRLs for GC/MS and LC/MS methods

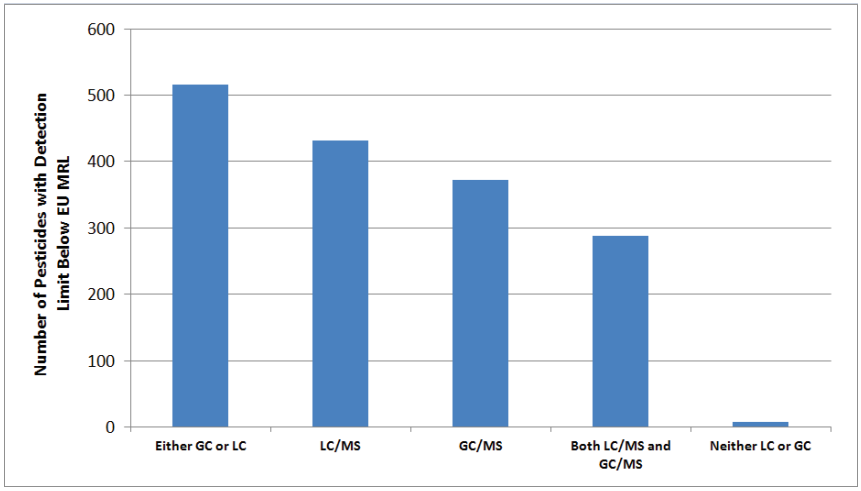


Benefits of Comprehensive GC/LC Methodology

By combining both GC and LC methodologies in a comprehensive screening methodology, 516 pesticides were detected below their MRLs for an onion matrix. This is 144 more than were detected below their MRLs for GC/MS methodology alone, and 84 more than by LC/MS alone. Only 8 pesticides had calculated detection limits for both GC/MS and LC/MS greater than their EU MRLs. On average, these 8 compounds" detection limits were four times their EU MRLs for the technique that gave them their lowest detection limit.

Furthermore, 288 compounds were able to be detected at concentrations below the EU MRL by both GC/MS and LC/MS methodology. This indicates that for a majority of these pesticides the two orthogonal techniques can be used together to increase confidence in the identification and quantitation. Figure 8 displayed below details these results.

FIGURE 8. Number of pesticides with detection limits below the EU MRL for GC/LC combined methodology compared with LC and GC methodology separately. Also displayed are numbers of pesticides detected below the MRL for both GC and LC methodology, and by neither methodology.



Conclusion

Methodology for both GC and LC/MS was developed and employed to analyze over 500 pesticides in a food matrix extracted with QuEChERS methodology. A summary of results, conclusions and possible future investigations for this project are as follow:

- 372 of 524 total pesticides were detected at levels under EU MRLs for onion samples by GC/MS
- 432 of 524 were detected at levels under EU MRLs for onion samples by LC/MS
- 516 of 524 were detected by either GC/MS, LC/MS, or by both GC/MS and LC/MS, demonstrating the power of combining these two techniques.
- For future work, a 10 µL large volume GC injection could be employed for the GC/MS methodology to better compare with the LC/MS methodology, and to try to lower the eight problematic pesticides detection limits under the EU MRL.
- Also, future work could explore techniques to selectively increase sensitivity for the eight problematic compounds, such as weighting SRM dwell time more heavily for these compounds, or decreasing resolution for these compounds, trading selectivity for sensitivity.

References

1. Steven J. Lehotay, Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERs) Approach for Determining Pesticide Residues. Methods in Biotechnology, 2006, 19, 239-261.

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Broad Scope Pesticide Screening in Food Using Triple Quadrupole GC-MS

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Overview

Purpose: To demonstrate two different ways to perform targeted and non-targeted screening of pesticides in one analytical run

Methods: Screening for 600 pesticides in selected reaction monitoring (SRM) mode or a smaller subset in selected reaction monitoring/ full scan (SRM/FS) mode

Results: Either method can be used to analyze targeted and non-targeted compounds with little loss of sensitivity

Introduction

The increased accessibility of high selectivity GC-MS has enabled more generic sample preparation in pesticide testing, allowing consolidation of multiple analyte lists and matrices into one method. GC-MS/MS is well suited to multi-residue analysis in a diverse range of matrices. However, as the number of targeted compounds increases, the complexity of method optimization increases and analytical performance becomes compromised. Furthermore, there is a desire to look beyond targeted lists for other potentially harmful food contaminants. Presented here is the use of smart instrument control and data processing software applied to GC-MS/MS analysis of 600 pesticides in matrix to mitigate analytical performance degradation through MS duty cycle optimization. Also discussed is the combining of this optimized targeted quantitation with general unknown analysis through full scan/SRM.

Method 1 – Screening For 600 Pesticides

Sample Preparation

Lettuce was purchased from a local grocery store and was extracted with 1:1 ethyl acetate/cyclohexane following the QuEChERS method of extraction and clean-up, then 5 mL of solvent exchanged into 1 mL of hexane:acetone (9:1). The concentrated extract was spiked with various mixes of calibration standards.

Gas Chromatography

The Thermo Scientific™ TRACE™ 1310 GC was equipped with both an SSL and PTV inlet. A 1 μL injection was performed on the PTV inlet. The liner was a Siltek™ deactivated baffled liner (Thermo Scientific part number 453T2120). Chromatographic separation was achieved by using a 5% diphenyl/95 % dimethyl polysiloxane column (30 m x 0.25 mm 0.25 μm). See Table 1 for the parameters for the PTV and oven.

TABLE 1. PTV and Oven Parameters.

PTV	Mode	Temp	Split Flow	Splitless Time	Purge Flow
	Splitless	75	50	2.00	5.00

Flow Ramps	Rate (mL/min)	Flow (ml/min)	Hold (min)
		1.2	30
	2	3	7.2

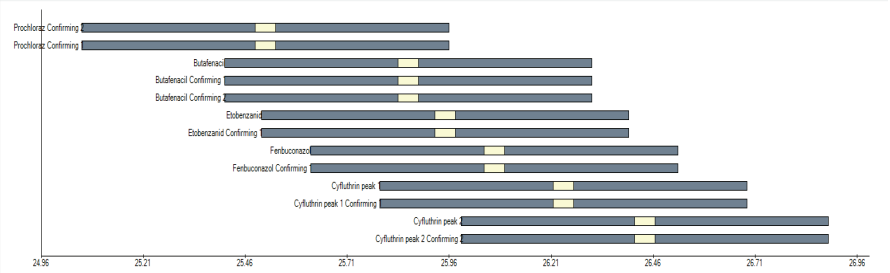
Injection phases	Pressure (kPa)	Rate (°C/sec)	Temp (°C)	Time (min)	Flow (mL/min)
Injection	70			0.1	50
Transfer	210	2.5	300	3.00	
Cleaning		14.5	330	20	75

Oven Program	Ramp	Rate (°C/min)	Temp (°C)	Hold Time (min)
	Initial		90	5
	1	25	180	0
	2	5	280	0
	3	10	300	5

Mass Spectrometry

The targeted screening using SRM of 600 compounds was performed using the Thermo Scientific™ TSQ™ 8000 triple quadrupole MS. After retention times were determined in full scan, a timed-SRM method using selected reaction monitoring (SRM) was constructed to analyze all compounds in a single injection. Over 1,300 transitions were entered into the method from the TSQ 8000 Pesticide Analyzer Compound Database. This automatically populated both the processing and instrument method through the TSQ 8000 system Method Synch. The transfer line was set to 250 C, and the ion source was at 300 C. Figure 1 demonstrates timed-SRM (t-SRM) which allows for the analysis of the 600 pesticides and provides for good sensitivity.

FIGURE 1. Small Section of Timed-SRM.



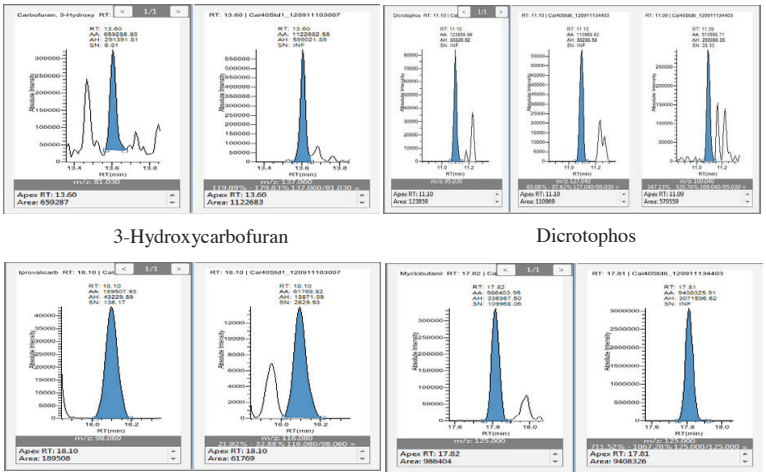
Results

Quantitative performance was determined for 52 pesticides in lettuce matrix during the screening for all 600 pesticides. The linearity for all of the compounds was $R^2 > 0.98$. Curves were generated using Thermo Scientific™ TraceFinder™ software. Ten replicates of a 40 ppb matrix spike sample were also analyzed. To test screening capability, a few additional compounds were added to the 40 ppb spike which had not been part of the calibration, but could be identified through the use of this method. The average concentration and %RSD of the 40 ppb standard are given in Table 2. Figure 5 shows the quantitation ions and confirming ions of the compounds in the 40 ppb spiked sample that were not a part of the original calibration. This demonstrates the ability of the method and the instrument to identify targeted compounds in samples for which the instrument is not calibrated.

TABLE 2. 40 ppb Standard Spiked into Lettuce Matrix.

Compound Name	Avg	%RSD		Avg	%RSD
Acibenzolar-S-methyl	32.1	8.8	Flutolanil	35.1	6.0
Azinphos-methyl	48.3	4.4	Fuberidazol	45.5	9.8
Azoxystrobin	39.5	2.3	Furalaxyl	62.4	4.4
Benalaxyl	43.8	6.3	Imazalil	45.6	3.5
Bendiocarb	50.7	3.9	Indoxacarb	47.2	9.2
Bitertanol	48.4	7.1	Isoprocarb	43.9	2.3
Boscalid (Nicobifen)	44.0	3.2	Mefenacet	47.1	2.9
Buprofezin	39.6	5.5	Metalaxyl	38.8	8.3
Carbaryl	56.1	2.3	Methiocarb	58.7	4.0
Carbofuran	45.1	11.8	Mevinphos	46.2	6.0
Carboxin	44.6	4.2	Oxadixyl	41.4	4.6
Carfentrazon-ethyl	39.1	5.4	Piperonyl butoxide	42.6	2.0
Clethodim	30.6	15.4	Pirimicarb	26.6	16.5
Cyprodinil	42.5	2.9	Propargite	55.9	6.5
Diethofencarb	41.2	6.7	Propham	40.2	1.7
Difenoconazole peak 1	53.7	3.0	Propiconazole peak 1	43.7	18.5
Difenoconazole peak 2	45.5	3.6	Propiconazole peak 2	49.3	6.0
Dimethomorph-1	52.8	7.1	Propoxur	46.9	2.1
Dimethomorph-2	49.7	3.2	Pyridaben	39.0	1.4
Ethofumesate	40.9	4.3	Pyrimethanil	37.5	15.3
Fenamidone	49.8	5.0	Spiromesifen	62.8	6.0
Fenbuconazol	40.7	1.2	Spiroxamine	52.3	7.0
Fenoxycarb	44.4	3.0	Thiabendazole	49.6	9.9
Flonicamid	44.7	6.1	Triazophos	46.7	4.3
Fludioxonil	45.2	5.7	Triflumizole	48.5	14.3
Flusilazole	44.8	6.1	Zoxamide	58.6	4.3

FIGURE 2. Pesticides Identified by Ion Ratio Not in the Targeted Calibration Curve. First Peak is the Quan Peak, and the Others are for Confirmation.

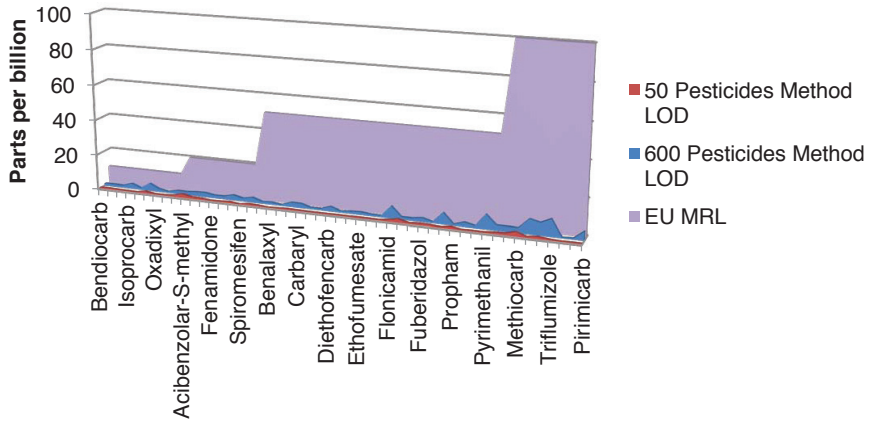


3-Hydroxycarbofuran Dicrotophos

Improvalicarb Myclobutanil

A second method was generated that targeted only the 52 compounds and contained only 104 transitions. Ten replicates of a 5 ppb and 10 ppb standard were analyzed to determine the MDLs for the two instrument methods, one with 1300+ transitions, and the other containing only 104 transitions. The results of compounds with MRLs for lettuce are shown in Figure 3. Although lower detection limits result from longer dwell times in the method with 104 transitions, the screening method that scans for 600 compounds is still capable of reaching the limits in lettuce set by the EU for the compounds requiring a targeted analysis in our list.

FIGURE 3. Comparison of MDLs: 52 Compounds vs. 600 Compounds.



Method 2 – Alternating SRM/FS

Sample Preparation and Gas Chromatography

The sample preparation and GC parameters remained the same as in the first study.

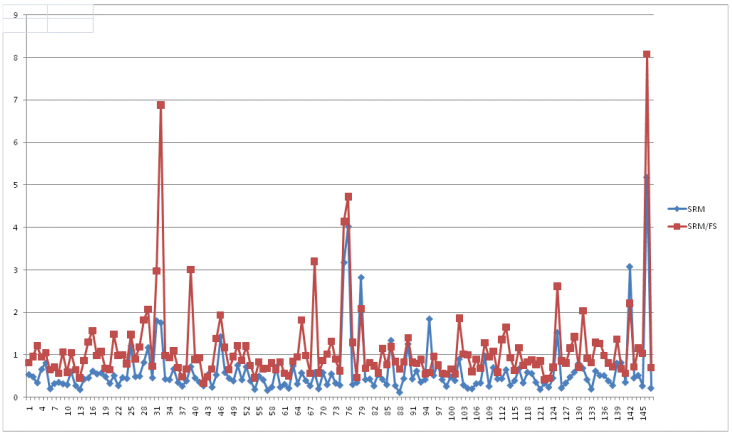
Mass Spectrometry

The scanning of 147 compounds was performed using the TSQ 8000 triple quadrupole MS. After retention times were determined in full scan, a timed-SRM method using selected reaction monitoring (SRM) was constructed to analyze all 147 compounds in a single injection. A second method was constructed, adding full scan to the analysis.

Results

A sample of fruit drink was extracted using the QuEChERS method of extraction and cleanup. The extract was concentrated 5x, then 147 pesticides were spiked into the extract to produce calibration curves from 1 ppb to 200 ppb. The calibration curves were constructed using TraceFinder software for both methods, SRM and alternating SRM/full scan for 147 pesticides. The linearity for most of the compounds was $R^2 >$ 0.98 for both methods of analysis. Ten replicates of a 1 ppb and 10 ppb standard in fruit juice extract were analyzed to determine the MDLs for the two instrument methods, SRM only and alternating SRM/full scan A comparison of the MDLs of both methods are shown in Figure 4. MDLs are slightly higher with the full scan added to the instrument method, but very comparable.

FIGURE 4. Comparison of MDLs from SRM vs. SRM/FS analysis (ppb).



Fruit drink was spiked at 100 ppb and analyzed using the SRM/FS instrument mode. This extract was also spiked with two phthalates at a 1 ppm level. The full scan chromatogram shows several peaks above the 100 ppb pesticide spike. Peaks are at retention times of 9.29, 9.73, 10.39, 10.91, and a very large saturated peak at 31.00 minutes. A close-up view of the first four compounds is shown in Figure 5. Figure 6 displays the NIST library matches for those non-targeted compounds.

FIGURE 5. Close-up View of Four Unknown Peaks in 100 ppb Spiked Fruit Drink.

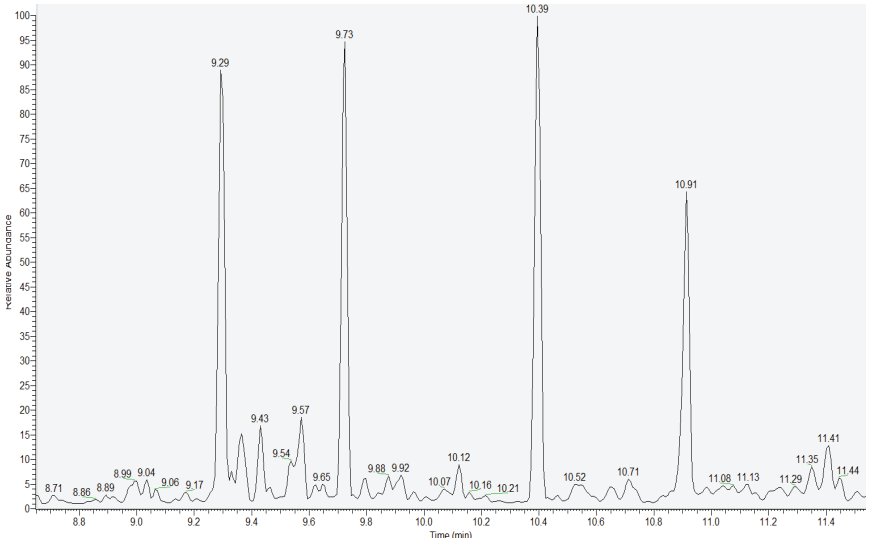
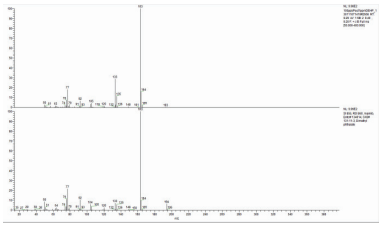
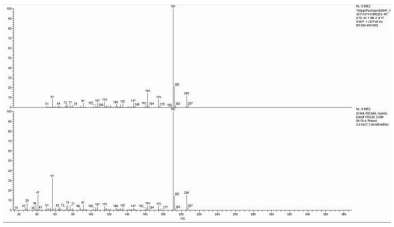


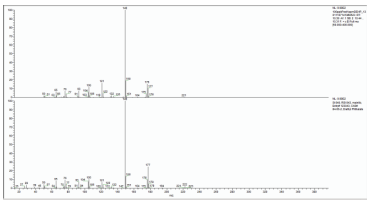
FIGURE 6. NIST Library Match for 4 Unknown Peaks.



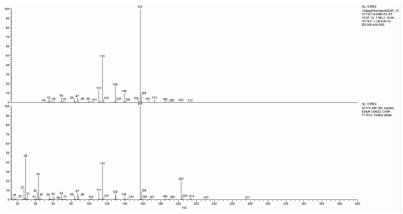
Dimethyl phthalate at 9.29 min



Phenol, 2,4-bis (1,1-dimethylethyl) at 9.72 min
used as a UV stabilizer in plastics



Diethyl phthalate at10.39 min



Triethyl citrate at 0.91 min
used as a plasticizer and food additive

Conclusion

Two different ways of analyzing targeted and non-targeted compounds have been demonstrated using the TSQ 8000 MS paired with the TRACE 1310 GC. Method 1 utilized the high SRM scan rate of the TSQ 8000 to scan for 600 pesticides in one analytical run without sacrificing sensitivity. Without having to calibrate all 600 pesticides, an analyst can still identify additional pesticides that may appear in the sample. Method 2 utilizes the ability of the TSQ 8000 to generate high quality library searchable full scan spectra at high scan speeds by operating the instrument in SRM/FS mode. This was done by selecting a number of target compounds for low level SRM analysis, while using full scan to identify unknowns of any classification, such as leachates from packaging, or nutritional compounds and preservatives added to food products.

Listed below is a summary of the two methods.

Screening for 600 Pesticides

- Screening for 600 pesticides without sacrificing sensitivity due to the high scan speed of the TSQ 8000
- 52 compounds calibrated with $R^2 > 0.98$
- Ability to identify pesticides not in the calibration through ion ratios
- Customizable compound list using AutoSRM feature to optimize new compounds

Alternating SRM/FS

- Target large number of compounds while collecting full scan data
- Quantitate targeted compounds while looking for non-targeted compounds
- Unknown identification of non-targeted compounds using the NIST library
- Calibration curves for most pesticides were $R^2 > 0.98$
- Comparable MDLs with or without full scan data collection
- Can be used for identifying contamination from packaging, nutritional components, or preservatives added to food products
- Customizable compound list using AutoSRM to optimize new compounds

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Validation of the Method for Determination of Pesticide Residues by Gas Chromatography – Triple-Stage Quadrupole Mass Spectrometry

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Thermo Fisher Scientific, Food Safety Response Center, Dreieich, Germany

Method 63899

Key Words
TraceFinder, TSQ, Chromatography, GC, GC-MS, Pesticide Residues, QuEChERS, Triple Quadrupole

1. Schematic of Method

Homogenization

Sample + IS

1. Weigh 10 g sample in 50 mL extraction tube

Extraction

2. Add 9.8 mL acetonitrile (20 mL water + 10 mL acetonitrile for wheat flour) and 200 µL stock IS
3. Shake for 10 min, centrifuge at 5000 rpm for 5 min

Clean up

4. Transfer supernatant into a 15 mL clean-up tube
5. Centrifuge samples at 5000 rpm for 5 min
6. Transfer supernatant into a GC vial
7. Add 50 µL sorbitol and 20 µL of injection standard

GC-MS/MS

2. Introduction

Pesticide residue analysis in food is one of the most important and challenging tasks in routine laboratory practice. The European legislation, which is currently the most strict legislation (European Regulation 396/2005 and Commission Directive 2006/125/EC), sets maximum residue limits (MRL) of pesticides in different products of plant and animal origin. This presents a significant analytical challenge with respect to the low limits of quantification (LOQ) required for some specified food matrices. A variety of GC and HPLC methods have been developed for multi-residue determination of pesticides employing a variety of sample preparation and cleanup techniques. In recent years the QuEChERS method has become widely adopted for preparing samples of fruit and vegetables, but the continuous need for more sensitive and accurate measurements requires new developments from the instrument producers as well.

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This method reports on in-house validation results and assessment of performance parameters of a complete multi-residue pesticide analysis method employing QuEChERS sample preparation kits, sample measurement by the newly developed Thermo Scientific™ TSQ™ 8000 Pesticide Analyzer system and rapid data analysis by Thermo Scientific™ TraceFinder™ software.

3. Scope

The objective of this validation study was to prove a complete workflow solution (delivered by Thermo Scientific chemicals, consumables and instrumentation) that can be implemented for routine multi-residue pesticide analysis (approximately 140 priority pesticides) in representative matrices (strawberry, wheat flour and leek). This was achieved in accordance with current legislation requirements, demonstrating that sensitivity of the assay conforms with the MRL values at the limits of detection (LOQ).[1-4]

4. Principle

Sub-portions of previously homogenized (for some instable compound cryogenic milling is recommended) samples were treated according to a standard QuEChERS method protocol (extraction and clean-up) prior to injection in the TSQ 8000 Triple-Stage Quadrupole GC-MS system.[5, 6]

Ready to use QuEChERS kit containing both extraction and clean-up tubes and associated protocol were used for sample preparation (Thermo Fisher Scientific, Runcorn UK). Identification of pesticide residues was based on retention time and ion-ratio confirmation using selective reaction monitoring (SRM) of characteristic transition ions, while quantification was calculated on matrix matched calibration and internal standardization. All method performance criteria were established according to the relevant guidelines.[1-4, 7]

5. Reagent List

		Part Number
5.1	Acetone, HPLC Grade	A/0606/17
5.2	Acetonitrile, LC-MS Grade	A/0638/17
5.3	Methanol, Optima LC-MS grade	A456-212
5.4	Toluene, HPLC grade	T/2200/08
5.5	Water, LC-MS grade	W/0112/17
5.6	Sorbitol, 500 g	10396733

6. Standard List

6.1 Pesticides

All individual pesticide compounds – Acephate, Acrinathrin, Amitraz, Azinphos-methyl, Azoxystrobin, Bifenthrin, Bitertanol, Boscalid, Bromopropylate, Bromuconazole, Bupirimate, Buprofezin, Cadusafos, Captan, Carbaryl, Carbofuran, Carboxin, Chlorfenapyr, Chlorfenvinphos, Chlorobenzilate, Chlorothalonil, Chlorpropham, Chlorpyrifos-ethyl, Chlorpyrifos-methyl, Cyfluthrin, Cyhalothrin, Cypermethrin, Cyproconazole, Cyprodinil, DDD, DDE,DDT, Deltamethrin, Demeton-S-methyl, Diazinon, Dichlofluanid, Dichloran, Dichlorbenzophenon, Dichlorvos, Dicofol, Difenoconazole, Dimethoate, Dimethomorph, Diphenylamine, Endosulfan, Endosulfan sulfate, EPN, Epoxiconazole, Ethion, Ethoprop (Ethoprophos), Etofenprox, Fenamiphos, Fenamiphos sulfone, Fenamiphos-sulfoxid, Fenarimol, Fenbuconazol, Fenitrothion, Fenoxycarb, Fenpropathrin, Fenpropidin, Fenpropimorph, Fenthion, Fenvalerate, Fipronil, Fludioxonil, Fluquinconazole, Flusilazole, Flutolanil, Flutriafol, Fluvalinate, Folpet, HCH alpha, HCH beta, HCH gamma Lindane, Hexaconazole, Imazalil, Iprodione, Isofenphos-methyl, Kresoxim-methyl, Linuron, Malathion, Mepanipyrim, Metalaxyl, Methacrifos, Methamidophos, Methidathion, Methiocarb, Metribuzin, Monocrotophos, Myclobutanil, Ortho-phenylphenol, Oxadiazon, Oxadixyl, Paclobutrazol, Paraoxon-methyl, Parathion (ethyl), Parathion-methyl, Pendimethalin, Permethrin, Phenthoate, Phosalone, Phosmet, Phosphamidon, Pirimicarb, Pirimicarb-p-desmetyl, Pirimiphos methyl, Prochloraz, Procymidone, Profenofos, Propargite, Propiconazole, Propyzamide, Prothiofos, Pyraclostrobin, Pyridaben, Pyrimethanil, Pyriproxyfen, Quinoxyfen, Spirodiclofen, Tebuconazole, Tebufenocide, Tebufenpyrad, Tefluthrin, Tetraconazole, Tetradifon, Tetrahydrophthalimide, Thiabendazole, Tolclofos-methyl, Tolyfluanid, Triadimefon, Triadimenol, Trifloxystrobin, Trifluralin, Triticonazole, Vinclozolin) were obtained from Sigma-Aldrich® (Germany) and Laboratory Instruments Srl (CASTELLANA GROTTE, Italy).

6.2 Internal standards

1-bromo-4-fluorobenzene (BFB), triphenylphosphate (TPP) (both from Sigma-Aldrich, Germany)

6.3 Quality Control Materials

FAPAS #19140QC (lettuce), FAPAS #19141QC (green bean) and FAPAS #19142QC (melon puree)

Note: FAPAS samples were selected primarily on content of target pesticides. However, due to limited availability, matrices are slightly different from the validated matrices.

7. Standards and Reagent Preparation

7.1 Individual Pesticide Standard Stock Solutions

Prepared gravimetrically in ~1000 mg/L concentration by weighing 10 mg from each analyte into a 20 mL amber screw cap vial on a five digit analytical balance and dissolving in 10 mL of appropriate solvent (acetone, toluene or acetonitrile depending on the individual compound). Concentrations of each individual standard stock solutions were calculated gravimetrically using weight of added compounds and solvents. All individual standard stocks were stored in a freezer at -20 °C. Validity of individual standard stock solutions was 6 months.

7.2 Intermediate Standard Stock and Working Standard Solutions

Prepared by pipetting the appropriate amount of each individual standard stock and diluting it with acetonitrile. The concentration of intermediate standard stock solutions was 5000 ng/mL. Working standards were prepared by diluting intermediate standard stock solution accordingly. Intermediate standard stock solutions were stored in a freezer at -20 °C, and the working solutions in a fridge at 4 °C. Validity of intermediate stock solutions was 3 months.

7.3 Individual Internal Standard Stock Solutions

Prepared gravimetrically in ~1000 mg/L concentration by weighing 10 mg from each analyte into a 20 mL amber screw cap vial on a five digit analytical balance and dissolving in 10 mL of acetone for TPP and 10 mL toluene for BFB. Exact concentration values were determined based on the gravimetrical values of both weighed compound and added solvent. Individual internal standard stock solutions were stored in a freezer at -20 °C. Validity of individual internal standard stock solutions was 6 months.

7.4 Working Internal Standard Stock Solutions

Prepared individually by pipetting the appropriate amount of each individual standard stock solution and diluting it with acetonitrile. The concentration of working internal standard stock solutions was 5000 ng/mL and was used for direct spiking of the samples. Validity of working stock solutions was 3 months.

7.5 1% Sorbitol Solution (Analyte Protectant)

Prepared in 70/30 v/v% ACN/H_2O and used for adding prior to injection. Protectant solution was added to the sample prior to injection in order to prevent undesired analyte interaction and consequent losses during the injection.[8]

8. Apparatus

		Part Number
8.1	Fisher precision balance	XP-1500FR
8.2	Sartorius analytical balance	ME235S
8.3	Thermo Barnstead EASYpure®II water	3125753
8.4	ULTRA-TURRAX® – G25 dispergation tool	1713300
8.5	ULTRA-TURRAX	3565000
8.6	Vortex shaker	3205025
8.7	Vortex universal cap	3205029
8.8	Horizontal shaker	1069-3391
8.9	Horizontal shaker plate	1053-0102
8.10	Thermo Heraeus Freco 17 micro centrifuge	3208590
8.11	Pesticide Analyzer (TSQ 8000 Triple Stage Qudrupole GC-MS with Thermo Scientific™ TRACE™ 1310)	

9. Consumables

		Part Number
9.1	GC vial kit	60180-599
9.2	Pipette Finnpipette 100–1000 µL	3214535
9.3	Pipette Finnpipette 10–100 µL	3166472
9.4	Pipette Finnpipette 500–5000 µL	3166473
9.5	Pipette holder	3651211
9.6	Pipette tips 0.5–250 µL, 500/box	3270399
9.7	Pipette tips 1–5 mL, 75/box	3270420
9.8	Pipette tips 100–1000 µL, 200/box	3270410
9.9	Spatula, 18/10 steel	3458179
9.10	Spatula, nylon	3047217
9.11	Centrifuge tube rack	1066-3721
9.12	QuEChERS extraction tube, 50 mL, 250 pack	60105-216
9.13	QuEChERS clean-up tube, 15 mL, 50 pack	60105-225
9.14	GC column Thermo Scientific™ TraceGOLD™ TG-5SilMS, 30 m × 0.25 × 0.25 mm	10177894
9.15	PTV Baffle Liner (Siltek), Deactivated, 2 mm ID × 2.75 mm OD × 120 mm Length	453T2120
9.16	2 mL vial rack	12211001

10. Glassware

		Part Number
10.1	Volumetric flask, 10 mL	FB50143
10.2	Volumetric flask, 25 mL	FB50147
10.3	40 mL screw cap vial	1054-1593
10.4	Caps for 40 mL screw cap vial	1009-0962
10.5	500 mL bottle	9653640
10.6	100 mL bottle	1006-8060

11. Procedure

11.1 Sample Preparation

Blank matrix samples (strawberry (SB), wheat flour (WF) and leek (LK)) used for validation experiments were purchased in local retail stores and were homogenized with an Ultra-Turrax homogenizer, extracted and cleaned-up prior to sample preparation. Matrix extracts were used as matrix blank samples and dilution solvents for matrix-matched calibration. Ready to use Thermo Scientific QuEChERS extraction kits were used for sample preparation, and contained 4 g $MgSO_4$, 1 g NaCl, 1 g trisodiumcitrate dehydrate and 0.5 g disodiumcitrate sesquihydrate for buffered extraction of target compounds. Pre-prepared clean-up tubes contained 1200 mg $MgSO_4$, 400 mg PSA and 400 mg C18 for increased clean-up efficiency for more complex matrices such as leek. The same QuEChERS protocol was applied for all of the matrices.

11.1.1 Homogenization of Matrices

11.1.1.1 Select larger amount of strawberry (~500 g) and bunch of leek matrices and put into an appropriate size beaker and label it.

11.1.1.2 Attach the G25 dispergation tool to the Ultra-Turrax homogenizer. (For better recovery for some unstable compounds cryogenic homogenization is advised).

11.1.1.3 Start homogenization at middle rotation speed (speed level 2–3) and continue to form a smooth homogenate.

11.1.2 Sample Extraction and Clean-up

11.1.2.1 Weigh 10 g sample into a 50 mL QuEChERS extraction tube containing 4 g $MgSO_4$, 1 g NaCl, 1 g trisodiumcitrate dehydrate and 0.5 g disodiumcitrate sesquihydrate.

11.1.2.2 Add 200 µL 5000 ng/mL internal standard #141 to the samples.

11.1.2.3 Add 10 mL ACN to SB and LK samples. For WF, first add 20 mL H_2O to the samples, let it completely wet the sample and then add 10 mL ACN to it.

11.1.2.4 Shake samples for 10 min on a horizontal shaker and centrifuge with 5000 rpm for 5 min. Transfer supernatant (~8 mL) into the 15 mL QuEChERS clean-up tubes containing 1200 mg $MgSO_4$, 400 mg PSA and 400 mg C18.

11.1.2.5 Vortex for 1 min and centrifuge samples with 5000 rpm for 5 min.

11.1.2.6 Collect supernatant and transfer 1 mL into a GC vial for instrumental analysis.

11.1.2.7 Add 50 µL sorbitol solution (protectant) and 20 µL 5000 ng/mL injection standard (BFB) to the GC vials prior to injection.

11.2 GC-MS/MS Analysis

Sample measurements were carried out using the TRACE 1310 gas chromatograph coupled to the TSQ 8000 Triple Stage Quadrupole Mass Spectrometer (Pesticide Analyzer). For instrument control, analysis, data review and reporting TraceFinder 3.1 software was used.

11.2.1 GC method settings

The injector settings were as follows:

Injector:	Thermo Scientific™ TriPlus RSH Autosampler with 10 µL injection syringe
Liner:	PTV Baffle Liner (Siltek), Deactivated, 2 mm ID × 2.75 mm OD × 120 mm Length (recommended to be changed after 40 injections of matrix samples)
Injection mode:	splitless PTV, basic mode
Carrier mode:	constant flow
Inlet temp:	75 °C
Split flow:	50 mL/min
Splitless time:	1 min
Injection volume:	1 µL
Plunger strokes:	3
Air filling mode:	auto
Carrier flow:	1.2 mL/min
PTV injection time:	0.1 min
PTV transfer rate:	2.5 °C/s
PTV transfer temp:	300 °C
PTV transfer time:	3 min
PTV cleaning rate:	14.5 °C
PTV cleaning temp:	330 °C
PTV cleaning time:	20 min
PTV cleaning flow:	75 mL/min
PTV cleaning phase:	post cycle temperature cool down

The GC oven settings were as follows:

Carrier gas:	1.2 mL/min Helium (constant flow)
PTV cleaning phase:	post cycle temperature cool down

Table 1. GC temperature programming

#	Rate [°C/min]	Temperature [°C]	Hold Time [min]
Initial		40	1.5
1	25	90	1.5
2	25	180	0
3	5	280	0
4	10	300	5

11.2.2 Triple Quadrupole MS Settings

Mass spectrometric detection was carried out using the TSQ 8000 triple-quadrupole mass spectrometer in timed-SRM mode. All method and SRM settings were taken from the Thermo Scientific TSQ 8000 Pesticide Analyzer system method.[6] Ion ratio values were revised and adapted for each investigated matrices.

The settings were as follows:

Scan type:	timed-SRM (details in Table 2)
Ionization:	EI +
MS transfer line temp:	250 °C
Ion source temp:	300 °C
Cycle time:	0.3 s
Minimum baseline peak width:	3 s
Desired scans per peak:	10
Minimum dwell time:	0.001 s
Q1 resolution:	normal (0.7 Da)

11.3 Calculation of Results

Internal standardization was applied for quantification of target pesticides. The relevant response factors (R_f) were defined by the equation below. Calculation of final result was performed using the following equations.

11.3.1 Equations

Calculation of the response factor:

$$R_f = \frac{A_{St} \times c_{[IS]}}{A_{[IS]} \times c_{St}}$$

R_f – the response factor

A_{St} – the area of the pesticide peak in the calibration standard

$A_{[IS]}$ – the area of the internal standard peak of the calibration standard

c_{St} – pesticide concentration of the calibration standard solution

$c_{[IS]}$ – the internal standard concentration of the calibration standard solution

Calculations of sample amount in each sample (the absolute amount of pesticide extracted from the sample):

$$X_{analyte} = \frac{A_{analyte} \times X_{IS}}{A_{IS} \times R_f}$$

$X_{analyte}$ – the absolute amount of pesticide that was extracted from the sample

$A_{analyte}$ – the area of pesticide peak in the sample

$A_{[IS]}$ – the area of the internal standard peak in the sample

$X_{[IS]}$ – the absolute amount of internal standard added to the sample

Calculations of sample amount in each sample (the absolute amount of pesticide extracted from the sample):

$$C = \frac{X_{analyte}}{m}$$

m – the weight of sample [g]

$X_{analyte}$ – absolute analyte amount [ng]

12. Method Performance Characteristics

In-house validation of the method was carried out on all matrices and target pesticides. European guidelines for single laboratory validation and pesticide residue analysis were used for establishing method performance criteria.[1, 2] All method performance parameters were compared to the relevant legislative requirements and maximum residue limit (MRLs).[2-4, 7] For compounds containing more isoforms, only one performance criteria was established.

12.1 Selectivity

Method (SRM) selectivity was assessed based on the presence of specific ion transitions (quantifier ion and two transitions for compound confirmation) at the corresponding retention time (Table 2), as well as the observed ion ratio values corresponding to those of the standards. Acceptance criteria for retention time and ion ratios were set according to current quality control criteria.[1, 3] Matrix blank samples were also inspected for the presence of interfering peaks in close vicinity of the target retention times for which (according to SANCO guidline definitions) <30% of LOQ acceptance criteria was applied.[3] Additional peaks in close vicinity of target peaks in blank samples were observed for chlorpropham (LK), demethon-s-methyl (SB), fenhexamide (WF, LK), fenitrothion (WF, LK), procymidon (WF), phosphalone (SB), permethrin (WF, LK), fenpropathrin (LK), o-phenylphenol (WF) and carbofuran (SB, WF). However, they were all clearly resolved by retention time from the target peaks (Rs>1.5) except carbofuran in SB and WF and propargite in WF and LK matrices.

12.2 Linearity, Response Factor, Matrix Effect

The calibration curves were created at six levels (matrix-matched) and injected in duplicate. R_f values for internal standardization were determined from the calibration curves for all matrices and internal standards by calculating cumulative average response factor over the whole calibration range. The linearity of calibration curves was assessed in three groups of compounds (depending on the relevant MRL values) in calibration ranges of 0–200, 0–1000 and 0–2000 ng/g, respectively, (details and results in Table 3). Calibration levels were equidistantly distributed over the calibration range. Linear function was evaluated according to Mandel's fitting test and plotting of residuals for which <20% acceptance limit was set.[3] Correlation coefficient values were additionally established for which an artificial 0.985 was set as an acceptance limit, as no legislative limits are defined for them. The set value wasn't met for fenpropathrin and dichlofluanid (LK) and propargite (WF) based on the high LOQ values related to the calibration levels. No weighted function was applied.

Matrix effects were evaluated by (Youden-) plotting of measured relative peak areas of calibration standards in solvent against the areas in the relevant matrix. No matrix effect is observed if the difference of the slope (dif%) of the fitted line is less than 20% from the ideal (y=x) curve, while matrix effects are observed when the difference is between 20–50% (minor matrix effect) or exceeds 50% (major matrix effect). Matrix effect results are listed in Table 3. For the compounds with demonstrated matrix effect application of matrix matched calibration is required.

12.3 Accuracy

Method trueness was assessed by recovery studies using blank matrices spiked at three concentration levels (L1, L2 and L3) and injected in six individually prepared replicates. (Table 4). Spiking of samples occurred prior to sample preparation. Found concentrations, recovery and relative standard deviation (% RSD) were calculated (Table 5). According to SANCO requirements recovery values are deemed acceptable if between 70–120%.[3] Values were calculated only for those cases in which spiking levels were higher than the compound LOQ in the particular matrix. Recovery values could not been established for amitraz in WF and captan, chlorthalonil and tolyfluanid in LK matrices due to the high LOQ values measured relative to the spiked levels. Strong influence of matrix on the results were observed in several cases and results could not been established at one or two spiking levels based on the measured different LOD/LOQ values in the different matrices (details in Table 4). For routine measurement these compounds in these matrices have to be measured with separate, specially optimized analytical methods. Method bias was established by means of external quality control materials obtained from FAPAS (York, UK). Available FAPAS materials were #19140QC (lettuce puree), #19141QC (green bean puree) and #19142QC (melon puree). The available Fapas samples represented only a limited number of the target compounds and different matrices from those targeted. However, measured values showed good agreement with the assigned values in all cases except carbofuran, in which the measured value was slightly below the acceptance range. This could be due to differences between the two different matrix characteristics. Details on the measured FAPAS values are listed in Table 7.

12.4 (Intermediate) Precision

Instrument injection precision was tested for both retention time and peak area for all target compounds by subsequent injections (n=6) of low concentration level (L1) standard solutions. Insturment injection precision for retention time was below 0.5% for all compounds and between 1.2–18.04% (fipronil and fenamiphos-sulfoxide) for peak area without internal standard compensation indicating reliable instrument performance. Method within-day and between-day precision values were determined for each matrix at middle spiking level (L2) and expressed as %RSD over 3 days with individually prepared samples (n=6). Mean within-day precision values were determined as an average of the 3 individual days' mean precision, while between-day precision was expressed as mean of the overall precision data. According to SANCO requirements <20% was set as acceptance criteria for the target compounds and matrices.[3] Measured values are shown in Table 5.

12.5 Limit of Detection, Limit of Quantification

Limits of detection and quantification were estimated following the IUPAC. Measured method LOD, LOQ and the relevant legislative limits (MRLs) are listed in Table 6.[7] An artificial MRL=10 ng/g was set as target value for compounds, for which no MRL values are legislatively defined. The expectation of the method was to meet MRL values at least at LOQ level which was achieved for the vast majority of target compounds. For methiocarb (WF, LK), carbofuran (SW), oxadyxil (WF) and propargite (WF, LK) the established LOQ values were below the targeted MRLs' value. However, with exchanging of quantifier and qualifier ions the target values can be reached. For fenpropathrin (WF, LK), amitraz (WF) and tebufenocid (all matrices), the target values could not be reached even when exchanging the quantifier and qualifier ions.

12.6 Robustness

A robustness study was performed by varying parameters like laboratory personnel, extraction and clean-up batches. Results were compared to the original method and significant differences were sought based on ANOVA analysis. None of the parameters which were varied led to significant differences in measured values, consequently indicating that the method was robust.

13. Conclusion

Full in-house validation of a complete method intended for routine pesticide residue measurements was carried out. The goal of the study was to obtain an objective and realistic overview of the analytical performance of a widely used and accepted sample preparation method combined with state of the art analytical instrumentation. The method performance parameters indicate that the performance for the majority of target compounds complies with current regulatory requirements. Independent, external quality control materials were additionally applied to improve confidence in the measurement results. In some cases method performance parameters could not be established or measured values fell outside of the targeted range due to individual properties of compounds or strong matrix influences on the analytical results. For those compounds (in the relevant matrix), individually optimised sample preparation (additional or special clean-up) and instrumental methods have to be applied. From a practical point of view (especially for instable or active compounds) the best performance can be achieved by replacing the liner (and septum) after 40–50 injections. Overall it can be concluded that the complete workflow solution offered by Thermo Fisher Scientific in conjuction with the newly developed TSQ 8000 GC-MS system delivers the required system performance for the target compounds especially regarding sensitivity, selectivity and recovery.

14. References

1. European Commission 2002/657/EC
2. European Commission 2006/125/EC
3. European Commission SANCO/12495/2011
4. European Commission 788/2012/EC
5. Fussell et al. (2007) *Food Additives & Contam*, 24:1247-1256
6. Thermo Scientific TSQ 8000 Pesticide Analyzer Brochure, https://static.thermoscientific.com/images/D22018~.pdf
7. EU Pesticides Database, http://ec.europa.eu/sanco_pesticides/public/index.cfm
8. Anastassiades et al. (2003) *J. Chromatogr A*, 1015:163-184

15. Annex

Tables and Figures

Table 2. Selectivity parameters for the target compounds

** retention times for all isomers ** internal standard compound*

Name	RT (min)	Quantifier Ion			Qualifier Ion 1			Qualifier Ion 2			Ion Ratio (for qualifier ion 1/ qualifier ion 2) [% of quant. ion]
		Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	
Acephate	9.36	95.5	65.4	8	136.0	42.1	8	136.0	94.0	12	0.2 / 99
Acrinathrin	24.33	181.0	152.0	22	208.1	180.9	8	289.0	93.1	8	110 / 52
Amitraz	24.03	121.0	106.1	10	131.9	117.1	16	161.9	132.0	8	85 / 78
Azinphos-methyl	23.29	132.0	77.0	12	160.0	50.9	34	160.0	77.0	16	55 / 120
Azoxystrobin	30.33	344.1	156.0	34	344.1	171.9	36	344.1	329.0	14	100 / 250
Bifenthrin	22.08	165.1	163.6	24	181.0	165.9	10	181.0	179.0	12	3800 / 400
Bitertanol	25.25	170.0	115.1	34	170.0	141.1	20	170.0	169.1	16	140 / 40
Boscalid (Nicobifen)	27.09	112.0	76.0	12	139.9	76.0	22	139.9	112.0	10	240 / 350
Bromopropylate	22.09	184.9	75.5	30	184.9	156.9	12	340.8	185.0	14	2500 / 600
Bromuconazole	21.87/ 22.6*	172.9	74.0	38	172.9	109.0	26	172.9	144.9	16	100 / 150
Bupirimate	18.08	208.1	140.1	12	208.1	165.0	12	273.1	193.2	8	260 / 60
Buprofezin	18.08	105.1	50.9	32	105.1	77.0	18	175.0	132.1	12	275 / 75
Cadusafos	11.5	159.0	96.9	16	159.0	130.9	8	213.0	89.1	12	550 / 15
Captan	16.35	149.0	70.0	20	149.0	78.8	14	149.0	105.0	6	120 / 130
Carbaryl	14.13	115.0	89.0	16	144.0	115.1	22	144.0	116.1	10	800 / 400
Carbofuran	11.98	149.1	77.0	24	149.1	121.1	8	164.0	149.1	8	120 / 120
Carboxin	18.11	87.0	43.0	6	143.0	43.0	16	143.0	87.0	8	200 / 100
Chlorfenapyr	18.37	136.9	102.0	12	248.9	112.0	24	248.9	137.1	18	45 / 30
Chlorfenvinphos	16.13	266.9	159.0	16	266.9	203.0	10	323.0	266.9	14	25 / 80
Chlorobenzilate	18.89	111.0	75.1	14	139.0	74.9	26	139.0	111.0	12	215 / 440
Chlorothalonil	12.72	228.8	168.0	8	265.8	133.0	36	265.8	170.0	24	350 / 160
Chlorpropham	11.17	171.0	127.0	8	213.0	127.0	14	213.0	171.0	6	65 / 45
Chlorpyrifos-ethyl	14.88	196.7	107.0	36	196.7	168.9	12	313.9	257.9	12	240 / 135
Chlorpyrifos-methyl	13.67	125.0	47.0	12	125.0	79.0	6	285.9	93.0	20	110 / 55
Cyfluthrin	26.67	163.0	65.1	26	163.0	91.1	12	163.0	127.1	6	100 / 25
Cyhalothrin	23.94	180.9	151.9	22	197.0	141.1	10	208.1	180.9	8	95 / 80
Cypermethrin	27.28/ 27.53/ 27.63/ 27.72*	163.0	91.1	12	163.0	127.1	6	180.9	152.1	20	100 / 50
Cyproconazole	18.53	222.0	82.1	10	222.0	89.3	38	222.0	125.0	20	35 / 210
Cyprodinil	15.85	224.1	196.9	20	224.1	208.0	18	225.1	209.7	16	500 / 40
DDD p,p	19.16	235.0	165.1	20	235.0	199.0	14	236.8	165.0	20	21 / 48
DDE p, p	17.85	246.0	176.1	28	317.8	246.0	20	317.8	248.0	18	28 / 30
DDT p,p	20.39	235.0	165.1	22	235.0	199.5	10	236.8	165.0	22	1.5 / 48
Deltamethrin	30.04	181.0	152.1	22	252.8	92.9	16	252.8	172.0	8	40 / 35
Demeton-S-methyl	10.91	88.0	59.8	6	109.0	79.0	6	141.9	79.0	12	10.1 / 25
Diazinon	12.51	137.1	54.1	20	137.1	84.1	12	179.1	121.5	26	170 / 10

Table 2 continued

** retention times for all isomers ** internal standard compound*

Name	RT (min)	Quantifier Ion			Qualifier Ion 1			Qualifier Ion 2			Ion Ratio (for qualifier ion 1/ qualifier ion 2) [% of quant. ion]
		Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	
Dichlofluanid	14.69	123.0	51.0	32	123.0	77.0	18	223.9	123.0	10	210 / 120
Dichloran	12.03	175.9	148.0	10	205.9	147.9	20	205.9	176.0	10	50 / 160
Dichlorbenzophe-non, p,p'-	16.61	139.0	110.9	15	249.9	139.0	10				0.3
Dichlorvos	8.10	109.0	79.0	6	185.0	93.0	12	186.9	93.0	12	60 / 16
Dicofol	24.18	111.0	74.9	12	139.0	111.0	12	251.0	139.0	15	460 / 160
Difenoconazole	29.51/ 29.62*	265.0	139.0	36	265.0	202.1	16	323.0	265.0	14	90 / 220
Dimethoate	11.92	87.0	42.1	10	93.0	63.0	8	125.0	79.0	8	70 / 55
Dimethomorph	30.51/ 31.00*	165.0	77.0	18	165.0	137.0	10	301.0	165.1	12	390 / 130
Diphenylamine	10.96	167.1	139.4	26	167.1	140.1	18	167.1	166.1	16	130 / 550
Endosulfan	17.19/ 19	194.7	125.0	22	194.7	159.4	8	240.6	205.9	14	140 / 120
Endosulfan sulfate	20.23	238.7	203.9	12	271.7	234.9	12	271.7	236.8	12	47 / 550
EPN	22.04	157.0	77.0	22	169.0	77.0	22	169.0	141.0	8	120 / 210
Epoxiconazole	21.34	165.0	138.0	8	192.0	111.0	22	192.0	138.0	12	150 / 300
Ethion	19.17	153.0	97.0	10	230.9	128.9	22				90
Ethoprop (Ethoprophos)	11.02	157.9	96.9	16	157.9	113.9	6	200.0	158.0	6	75 / 70
Etofenprox	27.66	163.1	77.1	32	163.1	107.1	16	163.1	135.1	10	300 / 350
Fenamiphos	17.39	154.0	139.0	10	216.9	202.0	12	303.1	195.2	8	85 / 50
Fenamiphos sulfone	21.74	320.0	213.9	14	320.0	249.1	18	320.0	292.1	8	95 / 420
Fenamiphos-sulfoxid	21.59	304.0	196.0	10	304.0	234.0	10				35
Fenarimol	24.16	139.0	74.9	26	139.0	111.0	14	219.0	107.0	10	185 / 80
Fenbuconazol	26.31	129.0	77.8	18	129.0	102.0	14	198.1	129.1	8	230 / /370
Fenitrothion	14.44	125.0	79.0	8	277.0	109.0	16	277.0	260.0	6	45 / 48
Fenoxycarb	22.19	116.0	44.1	16	116.0	88.0	8	255.1	186.1	10	460 / 60
Fenpropathrin	22.39	97.1	55.1	6	181.0	126.8	28	181.0	151.9	22	22 / 92
Fenpropidin	14.38	98.2	41.5	18	98.2	55.1	14	98.2	70.0	10	1650 / 1850
Fenpropimorph	15.06	128.1	41.7	24	128.1	70.1	12	128.1	110.1	8	400 / 300
Fenthion	14.98	245.3	125.0	12	278.0	109.0	18	278.0	169.0	14	1300 / 500
Fenvalerate	28.73	125.0	89.0	18	167.0	89.0	32	167.0	125.0	10	45 / 300
Fipronil	15.96	366.9	212.9	28	366.9	244.9	20	368.8	214.9	30	30 / 65
Fludioxonil	17.61	153.7	127.0	8	248.0	127.0	26	248.0	153.8	18	290 / 160
Fluquinconazole	25.61	340.0	108.1	36	340.0	298.0	16	340.0	313.0	14	160 / 65
Flusilazole	18.05	206.0	151.3	14	233.0	151.9	14	233.0	164.9	16	230 / 350
Flutolanil	17.47	173.0	95.0	28	173.0	145.0	14	281.0	173.0	10	350 / 56
Flutriafol	17.31	123.0	75.0	24	123.0	95.0	12	219.0	123.0	12	180 / 72

Table 2 continued

** retention times for all isomers ** internal standard compound*

Name	RT (min)	Quantifier Ion			Qualifier Ion 1			Qualifier Ion 2			Ion Ratio (for qualifier ion 1/ qualifier ion 2) [% of quant. ion]
		Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	
Fluvalinate	29.03/ 29.16*	180.8	152.1	22	250.0	55.1	16	250.0	199.9	18	45 / 35
Folpet	16.54	104.0	76.0	10	130.0	102.0	12	259.9	130.1	14	92 / 62
HCH alpha	11.71	216.9	180.9	8	218.8	182.9	8				95
HCH beta	12.19	216.9	180.9	8	218.8	182.9	8				90
HCH gamma_ Lindane	12.39	216.9	180.9	8	218.8	182.9	8				100
Hexaconazole	17.54	213.9	123.5	28	213.9	159.0	18	231.0	175.0	10	950 / 1100
Imazalil	17.58	172.8	109.0	26	174.7	147.0	16	215.0	173.0	8	90 / 130
Iprodione	21.77	314.0	245.0	10	315.7	247.0	10	315.7	273.0	8	50 / 22
Isofenphos-methyl	15.65	199.0	65.0	34	199.0	121.0	10	241.1	121.1	20	395 / 70
Kresoxim-methyl	18.12	116.0	62.9	24	116.0	89.0	14	130.9	130.1	10	324 / 102
Linuron	14.63	159.8	133.0	12	187.0	124.0	20	248.0	61.1	8	70 / 120
Malathion	14.68	92.8	63.0	8	125.0	79.0	8	173.1	99.0	12	110 / 300
Mepanipyrim	17.21	222.0	206.0	26	222.0	207.1	14	223.1	207.4	24	220 / 41
Metalaxyl	14.01	131.9	117.0	12	160.1	130.0	18	160.1	144.8	10	100 / 80
Methacrifos	9.8	125.0	79.0	8	180.0	93.0	10	240.0	180.0	10	55 / 40
Methamidophos	8.03	141.0	64.0	18	141.0	79.0	20	141.0	94.8	8	420 / 520
Methidathion	16.7	145.0	58.0	14	145.0	85.0	6	302.6	284.9	14	370
Methiocarb	14.98	153.0	45.0	12	153.0	109.1	6	168.1	153.0	10	225 / 554
Metribuzin	13.67	198.0	55.0	26	198.0	82.1	16	198.0	110.0	10	300 / 100
Monocrotophos	11.4	96.9	82.0	10	127.0	95.0	16	127.0	109.0	10	105 / 350
Myclobutanil	17.98	179.0	90.0	28	179.0	125.0	14	179.0	151.7	8	320 / 60
Ortho-phenyl-phenol	10.09	141.1	115.1	14	170.1	115.0	34	170.1	141.1	22	91 / 100
Oxadiazon	17.87	174.9	76.0	28	174.9	112.0	12	174.9	147.2	6	226 / 52
Oxadixyl	19.12	131.9	117.0	16	163.1	117.0	24	163.1	132.1	8	110 / 260
Paclobutrazol	16.97	125.0	89.0	18	236.0	125.0	12	236.0	167.0	10	290 / 90
Paraoxon-methyl	12.83	95.9	65.0	12	109.0	79.0	6	230.0	105.9	16	140 / 110
Parathion (ethyl)	15.07	109.0	81.0	10	124.9	97.0	6	291.0	109.0	12	75 / 48
Parathion-methyl	13.85	124.9	47.0	12	124.9	79.0	6	263.0	109.0	12	105 / 60
Pendimethalin	15.81	252.1	161.0	14	252.1	162.0	8	252.1	191.3	8	130 / 85
Permethrin	25.38/ 25.64*	163.0	91.1	12	183.1	153.0	12	183.1	168.0	12	100 / 105
Phenthoate	16.25	121.0	77.0	22	246.0	121.0	8	274.0	121.0	10	100 / 120
Phosalone	23.15	121.1	65.0	10	182.0	74.8	30	182.0	111.0	14	105 / 190
Phosmet	21.89	160.0	50.9	38	160.0	76.9	22	160.0	133.0	10	170 / 110
Phosphamidon	13.47	127.0	94.9	16	127.0	109.0	12	264.1	127.0	12	380 / 100
Pirimicarb	13.08	166.1	55.0	18	166.1	96.0	12	238.1	166.1	10	120 / 230
Pirimicarb-p-desmetyl	13.36	152.1	42.0	25	152.1	96.0	10	224.1	152.1	10	230 / 120
Pirimiphos methyl	14.37	290.1	125.0	20	290.1	233.0	8	305.1	180.1	8	60 / 70
Prochloraz	25.74	69.9	42.0	8	180.1	138.1	12	308.0	147.1	12	160 / 10
Procymidone	16.4	95.9	53.0	16	95.9	67.1	8	283.0	96.1	8	400 / 65

Table 2 continued

** retention times for all isomers ** internal standard compound*

Name	RT (min)	Quantifier Ion			Qualifier Ion 1			Qualifier Ion 2			Ion Ratio (for qualifier ion 1/ qualifier ion 2) [% of quant. ion]
		Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	
Profenofos	17.73	296.7	268.9	10	336.9	266.9	12	336.9	308.9	8	190 / 35
Propargite	20.97	135.1	77.1	26	135.1	107.1	12	150.1	135.1	8	310 / 110
Propiconazole	20.19/ 20.39*	172.9	74.0	38	172.9	109.0	26	172.9	145.0	16	110 / 155
Propyzamide	12.5	172.9	74.0	38	172.9	109.0	26	172.9	145.0	14	105 / 190
Prothiofos	17.57	266.7	220.9	18	266.7	238.9	8	308.9	239.0	14	142 / 160
Pyraclostrobin	28.89	132.0	51.1	35	132.0	77.0	20	164.0	132.1	10	230 / 220
Pyridaben	25.62	147.1	117.1	20	147.1	119.1	8	147.1	132.1	12	55 / 58
Pyrimethanil	12.66	198.1	117.9	30	198.1	157.6	18	198.1	182.9	14	10 / 120
Pyriproxyfen	23.54	136.1	78.0	20	136.1	96.0	10	226.1	186.1	12	90 / 10
Quinoxyfen	20.18	237.0	208.0	26	271.8	237.1	12	307.0	237.0	18	55 / 33
Spirodiclofen	25.09	156.9	73.0	20	156.9	86.7	32	312.2	259.0	8	60 / 105
Tebuconazole	20.85	125.0	89.0	16	125.0	99.0	16	250.0	125.0	20	50 / 110
Tebufenocide	22.58	145.1	117.0	10	160.1	145.1	12				8
Tebufenpyrad	22.58	276.1	171.0	10	318.1	131.1	14	318.1	145.1	14	43 / 31
Tefluthrin	12.79	177.0	127.0	14	177.0	137.0	16	197.0	141.1	10	34 / 40
Tetraconazole	15.18	100.9	51.0	10	159.0	123.4	16	336.0	204.0	28	8 / 100
Tetradifon	22.97	159.0	74.8	32	159.0	111.0	20	159.0	131.0	10	125 / 252
Tetrahydroph-thalimide (THPI)	9.96	151.0	77.1	30	151.0	79.9	6	151.0	122.1	8	140 / 80
Thiabendazole	16.36	174.0	103.0	18	174.0	130.1	10	201.0	174.0	14	110 / 700
Tolclofos-methyl	13.86	265.0	219.9	20	265.0	250.0	12	266.8	252.0	12	285 / 80
Tolyfluanid	16.1	137.0	65.1	28	137.0	91.1	18	238.0	137.0	10	150 / 110
Triadimefon	15.17	208.0	111.0	20	208.0	126.7	12	208.0	180.8	8	65 / 120
Triadimenol	16.39	112.0	57.6	8	128.0	65.0	18	168.2	70.0	10	
Trifloxystrobin	20.16	116.1	63.0	24	116.1	89.0	14	145.0	95.0	14	295 / 40
Trifluralin	11.17	306.1	159.7	20	306.1	206.0	10	306.1	264.1	8	150 / 900
Triphenylphos-phate (TPP)**	21.01	215.0	168.1	16	326.1	168.6	28	326.1	325.3	10	6 / 62
Triticonazole	23.17	217.0	167.0	18	235.1	181.9	12	235.1	217.1	8	92 / 120
Vinclozolin	13.73	241.1	58.1	12	241.1	184.1	10	284.9	269.9	12	160

Table 3. Linearity and matrix effect results *(see text 12.2 for details on Youden plot slope results).* – *residue plot RSD% <20%* – *residue plot RSD% >20%*

Compound	Calibration Range [ng/g]	Strawberry			Wheat Flour			Leek		
		r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]
Acephate	0-200	0.9998		12	0.9995		9	0.9998		35
Acrinathrin	0-200	0.9976		9	0.9985		270	0.9976		61
Amitraz	0-2000	0.9884		39	n.d.	n.d.	n.d.	0.9920		38
Azinphos-methyl	0-1000	0.9885		20	0.9956		0	0.9890		52
Azoxystrobin	0-1000	0.9911		24	0.9979		130	0.9918		63
Bifenthrin	0-200	0.9997		10	0.9939		12	0.9947		24
Bitertanol	0-200	0.9993		24	0.9956		67	0.9986		18
Boscalid (Nicobifen)	0-200	0.9983		16	0.9946		61	0.9976		8
Bromopropylate	0-200	0.9986		9	0.9908		2	0.9988		19
Bromuconazole	0-200	0.9989		6	0.9965		7	0.9994		17
Bupirimate	0-1000	0.9970		5	0.9981		3	0.9995		21
Buprofezin	0-1000	0.9993		16	0.9984		13	0.9961		31
Cadusafos	0-200	1.0000		3	0.9997		14	0.9970		27
Captan	0-200	0.9963		63	0.9967		56	n.d.	n.d.	n.d.
Carbaryl	0-1000	0.9995		54	0.9991		50	0.9833		68
Carbofuran	0-200	0.9987		11	0.9907		31	0.9816		64
Carboxin	0-200	0.9989		6	0.9988		16	0.9998		18
Chlorfenapyr	0-1000	0.9991		16	0.9971		18	0.9994		35
Chlorfenvinphos	0-200	0.9996		9	0.9958		97	0.9982		10
Chlorobenzilate	0-200	0.9999		2	0.9971		5	0.9991		17
Chlorothalonil	0-200	0.9952		77	0.9991		25	n.d.	n.d.	n.d.
Chlorpropham	0-200	0.9999		1	0.9997		11	0.9971		18
Chlorpyrifos-ethyl	0-200	0.9998		11	0.9995		6	0.9994		22
Chlorpyrifos-methyl	0-200	0.9998		25	0.9995		32	0.9991		39
Cyfluthrin	0-200	0.9995		4	0.9918		130	0.9899		5
Cyhalothrin	0-200	0.9979		15	0.9972		39	0.9973		16
Cypermethrin	0-200	0.9993		10	0.9947		105	0.9900		15
Cyproconazole	0-200	0.9994		17	0.9975		29	0.9997		2
Cyprodinil	0-200	0.9594		5	0.9970		5	0.9993		10
DDD p,p	0-200	0.9984		4	0.9982		20	0.9987		7
DDE p, p	0-200	0.9999		11	0.9985		21	0.9983		9
DDT p,p	0-200	0.9974		21	0.9963		26	0.9926		18
Deltamethrin	0-200	0.9994		7	0.9935		149	0.9911		40
Demeton-S-methyl	0-1000	0.9997		0	0.9994		2	0.9995		6
Diazinon	0-200	0.9998		18	0.9996		23	0.9928		36
Dichlofluanid	0-1000	0.9962		6	0.9997		10	0.7016		99
Dichloran	0-200	0.9996		7	0.9993		21	0.9994		25
Dichlorbenzophenon, p,p'-	0-200	0.9976		24	0.9988		65	0.9904		99
Dichlorvos	0-200	0.9996		15	0.9992		37	0.9993		20
Dicofol	0-200	0.9989		2	0.9952		11	0.9991		20
Difenoconazole	0-200	0.9989		13	0.9965		225	0.9995		51
Dimethoate	0-200	0.9996		17	0.9997		4	0.9996		20

Table 3 continued

– residue plot RSD% <20% *– residue plot RSD% >20%*

Compound	Calibration Range [ng/g]	Strawberry			Wheat Flour			Leek		
		r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]
Dimethomorph	0-200	0.9995		37	0.9996		181	0.9984		42
Diphenylamine	0-200	0.9999		12	0.9994		24	0.9969		22
Endosulfan	0-1000	0.9994		7	0.9961		4	0.9969		17
Endosulfan sulfate	0-200	0.9988		4	0.9920		2	0.9980		20
EPN	0-200	0.9960		2	0.9947		56	0.9926		0
Epoxiconazole	0-200	0.9992		5	0.9966		14	0.9994		8
Ethion	0-200	0.9977		11	0.9967		21	0.9995		3
Ethoprop (Ethoprophos)	0-200	0.9998		7	0.9997		16	0.9978		17
Etofenprox	0-200	0.9985		15	0.9939		60	0.9986		3
Fenamiphos	0-200	0.9996		2	0.9992		40	0.9999		41
Fenamiphos sulfone	0-200	0.9968		16	0.9981		74	0.9933		25
Fenamiphos-sulfoxid	0-2000	0.9907		10	0.9940		101	0.8709		44
Fenarimol	0-200	0.9979		2	0.9958		8	0.9987		22
Fenbuconazol	0-200	0.9990		7	0.9949		33	0.9991		6
Fenitrothion	0-200	0.9994		15	0.9993		15	0.9992		23
Fenoxycarb	0-200	0.9990		9	0.9970		52	0.9989		4
Fenpropathrin	0-200	0.9981		7	0.9972		45	0.9146		6
Fenpropidin	0-1000	0.9998		18	0.9997		7	0.9962		17
Fenpropimorph	0-200	0.9998		10	0.9997		5	0.9943		27
Fenthion	0-200	0.9987		17	0.9998		21	0.9997		5
Fenvalerate	0-200	0.9999		10	0.9949		84	0.9973		19
Fipronil	0-200	0.9998		8	0.9984		26	0.9991		29
Fludioxonil	0-200	0.9800		1	0.9979		11	0.9992		23
Fluquinconazole	0-200	0.9976		22	0.9990		153	0.9995		39
Flusilazole	0-200	0.9984		2	0.9953		13	0.9977		11
Flutolanil	0-200	0.9989		15	0.9996		38	0.9997		7
Flutriafol	0-200	0.9996		1	0.9991		14	0.9996		23
Fluvalinate	0-200	0.9995		20	0.9956		131	0.9938		1
Folpet	0-2000	0.9959		76	0.9984		48	n.d.	n.d.	n.d.
HCH alpha	0-200	0.9999		8	0.9951		8	0.9977		15
HCH beta	0-200	0.9999		14	0.9993		16	0.9981		29
HCH gamma_Lindane	0-200	0.9999		12	0.9945		17	0.9961		21
Hexaconazole	0-1000	0.9938		8	0.9995		11	0.9999		11
Imazalil	0-1000	0.9987		14	0.9985		14	0.9998		26
Iprodione	0-200	0.9981		5	0.9984		34	0.9917		13
Isofenphos-methyl	0-200	0.9996		6	0.9996		54	0.9992		6
Kresoxim-methyl	0-200	0.9990		15	0.9974		15	0.9992		35
Linuron	0-1000	0.9986		50	0.9967		55	0.9996		42
Malathion	0-200	0.9985		14	0.9995		11	0.9816		30
Mepanipyrim	0-200	0.9993		24	0.9928		38	0.9995		11
Metalaxyl	0-1000	0.9999		20	0.9996		30	0.9980		37
Methacrifos	0-200	0.9994		3	0.9983		16	0.9951		19

Table 3 continued

– residue plot RSD% <20% *– residue plot RSD% >20%*

Compound	Calibration Range [ng/g]	Strawberry			Wheat Flour			Leek		
		r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]
Methamidophos	0-200	0.9995		0	0.9995		9	0.9967		33
Methidathion	0-200	0.9984		13	0.9997		14	0.9988		32
Methiocarb	0-2000	0.9988		2	0.9963		20	0.9876		33
Metribuzin	0-1000	0.9997		21	0.9996		22	0.9995		28
Monocrotophos	0-1000	0.9997		36	0.9990		11	0.9982		45
Myclobutanil	0-200	0.9994		2	0.9979		8	0.9991		20
Ortho-phenylphenol	0-200	0.9999		4	0.9995		18	0.9945		24
Oxadiazon	0-200	0.9999		8	0.9968		11	0.9956		28
Oxadixyl	0-200	0.9997		5	0.9969		4	0.9989		25
Paclobutrazol	0-200	0.9996		4	0.9997		1	0.9988		15
Paraoxon-methyl	0-1000	0.9957		40	0.9964		27	0.9875		43
Parathion (ethyl)	0-1000	0.9968		7	0.9956		4	0.9964		20
Parathion-methyl	0-200	0.9996		24	0.9985		30	0.9997		35
Pendimethalin	0-200	0.9950		15	0.9910		121	0.9937		75
Permethrin	0-200	0.9951		27	0.9961		70	0.9970		13
Phenthoate	0-1000	0.9991		18	0.9989		25	0.9996		32
Phosalone	0-200	0.9976		2	0.9921		33	0.9939		12
Phosmet	0-200	0.9972		28	0.9961		34	0.9922		61
Phosphamidon	0-200	0.9989		42	0.9997		37	0.9961		70
Pirimicarb	0-200	0.9998		16	0.9997		22	0.9990		32
Pirimicarb-p-desmetyl	0-1000	0.9999		26	0.9998		28	0.9994		36
Pirimiphos methyl	0-200	0.9987		15	0.9980		4	0.9986		25
Prochloraz	0-1000	0.9924		9	0.9974		37	0.9925		12
Procymidone	0-200	0.9999		17	0.9996		6	0.9969		26
Profenofos	0-200	0.9988		2	0.9992		>200	0.9940		34
Propargite	0-200	0.9991		9	0.8967		17	0.9997		51
Propiconazole	0-200	0.9986		13	0.9976		15	0.9877		10
Propyzamide	0-200	0.9999		9	0.9995		14	0.9946		25
Prothiofos	0-200	0.9993		20	0.9987		80	0.9986		4
Pyraclostrobin	0-200	0.9997		6	0.9954		56	0.9964		1
Pyridaben	0-200	0.9961		29	0.9967		79	0.9953		14
Pyrimethanil	0-200	0.9999		13	0.9997		13	0.9963		20
Pyriproxyfen	0-200	0.9982		1	0.9964		12	0.9996		17
Quinoxyfen	0-200	0.9977		15	0.9979		28	0.9998		2
Spirodiclofen	0-200	0.9995		7	0.9974		8	0.9950		34
Tebuconazole	0-200	0.9995		17	0.9969		22	0.9986		3
Tebufenocide	0-1000	0.9980		11	0.9975		34	0.9984		12
Tebufenpyrad	0-200	0.9987		8	0.9996		126	0.9996		4
Tefluthrin	0-200	1.0000		14	0.9994		20	0.9929		31
Tetraconazole	0-1000	0.9997		17	0.9997		13	0.9975		33
Tetradifon	0-200	0.9998		10	0.9959		11	0.9989		30
Tetrahydrophthalimide	0-200	0.9645		106	0.9638		51	0.8388		93

Table 3 continued

– residue plot RSD% <20% *– residue plot RSD% >20%*

Compound	Calibration Range [ng/g]	Strawberry			Wheat Flour			Leek		
		r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]
Thiabendazole	0-1000	0.9987		9	0.9996		8	0.9998		28
Tolclofos-methyl	0-200	0.9998		27	0.9990		57	0.9987		6
Tolyfluanid	0-1000	0.9970		6	0.9989		47	n.d.	n.d.	n.d.
Triadimefon	0-1000	0.9987		7	0.9996		8	0.9995		22
Triadimenol	0-1000	0.9993		2	0.9991		8	0.9992		26
Trifloxystrobin	0-200	0.9985		17	0.9978		61	0.9994		3
Trifluralin	0-200	0.9913		311	0.9973		62	0.9821		30
Triticonazole	0-200	0.9977		27	0.9975		70	0.9983		20
Vinclozolin	0-200	0.9996		18	0.9983		22	0.9973		27

Table 4: Recovery values [%] at 10 ng/g (level 1), 20 ng/g (level 2) and 100 ng/g (level 3) spike levels.

** spiking levels are 50, 100 & 500 ng/g* *** spiking levels are 100, 200 & 1000 ng/g* *<LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry			Wheat Flour			Leek		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
Acephate	84	88	63	68	75	60	68	72	56
Acrinathrin	100	79	67	121	118	85	129	69	24
Amitraz**	98	79	57	n.d.	n.d.	n.d.	126	95	69
Azinphos-methyl*	127	102	79	101	128	99	126	88	68
Azoxystrobin*	101	87	67	111	123	95	78	88	82
Bifenthrin	101	104	73	94	117	76	94	108	84
Bitertanol	101	109	82	116	118	81	82	109	88
Boscalid (Nicobifen)	93	101	81	111	116	83	111	111	86
Bromopropylate	92	109	90	117	114	82	97	111	89
Bromuconazole	87	106	90	108	114	79	88	106	88
Bupirimate*	83	111	101	105	113	83	93	120	99
Buprofezin*	82	112	97	100	112	80	100	125	97
Cadusafos	78	109	88	96	111	85	68	111	95
Captan	74	42	71	42	32	66	n.d.	n.d.	n.d.
Carbaryl*	106	81	65	110	100	71	83	76	72
Carbofuran	87	99	85	106	133	107	<LOQ	54	43
Carboxin	96	107	94	99	100	80	83	107	89
Chlorfenapyr*	86	112	100	104	118	83	84	118	99
Chlorfenvinphos	101	110	89	105	119	91	84	98	79
Chlorobenzilate	87	114	94	115	123	73	85	123	97
Chlorothalonil	133	73	36	76	56	62	n.d.	n.d.	n.d.
Chlorpropham	84	113	94	87	109	86	73	118	100
Chlorpyrifos-ethyl	86	110	87	95	113	88	91	132	100
Chlorpyrifos-methyl	114	112	80	100	121	95	93	135	103
Cyfluthrin	102	103	77	127	114	73	119	98	67
Cyhalothrin	103	85	79	117	118	86	104	77	65

Table 4 continued

** spiking levels are 50, 100 & 500 ng/g ** spiking levels are 100, 200 & 1000 ng/g <LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry			Wheat Flour			Leek		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
Cypermethrin	84	86	73	181	136	84	112	112	80
Cyproconazole	83	103	88	111	112	80	73	107	89
Cyprodinil	21	30	24	106	109	81	84	120	92
DDD p,p	96	105	86	88	108	85	79	120	100
DDE p, p	76	104	85	89	100	75	80	121	96
DDT p,p	97	94	68	124	141	120	82	118	90
Deltamethrin	100	77	56	114	107	70	93	84	58
Demeton-S-methyl*	93	106	84	97	111	93	96	122	92
Diazinon	87	113	91	95	110	86	77	125	101
Dichlofluanid*	110	72	62	37	48	73	<LOD	<LOD	55
Dichloran	83	109	95	106	120	92	78	116	90
Dichlorbenzophenon, p,p'-	77	104	86	<LOD	<LOQ	84	<LOQ	105	103
Dichlorvos	89	122	92	98	118	112	98	112	85
Dicofol	86	98	85	114	114	80	83	103	85
Difenoconazole	93	104	80	101	113	90	66	87	69
Dimethoate	86	95	82	79	113	95	94	117	86
Dimethomorph	92	99	73	90	124	114	86	102	81
Diphenylamine	102	107	74	56	70	79	75	122	95
Endosulfan*	86	101	78	114	121	67	76	118	97
Endosulfan sulfate	102	109	87	126	129	86	114	122	95
EPN	121	113	84	134	123	96	122	122	85
Epoxiconazole	103	116	88	109	119	86	89	116	95
Ethion	112	110	84	116	120	86	77	116	97
Ethoprop (Ethoprophos)	91	99	73	99	111	89	72	114	97
Etofenprox	91	101	79	119	114	78	89	103	82
Fenamiphos	90	103	92	68	84	71	75	103	87
Fenamiphos sulfone	106	95	66	119	117	92	63	51	57
Fenamiphos-sulfoxid**	144	150	117	119	137	131	65	89	91
Fenarimol	95	100	79	111	115	79	85	101	83
Fenbuconazol	100	110	85	123	123	85	92	113	92
Fenitrothion	105	102	83	107	123	94	111	129	96
Fenoxycarb	98	103	85	114	120	89	97	112	91
Fenpropathrin	86	105	91	<LOD	<LOD	82	<LOD	<LOD	77
Fenpropidin*	35	36	23	43	29	26	n.d.	9	20
Fenpropimorph	59	79	65	68	79	62	40	80	73
Fenthion	87	100	108	61	84	77	108	122	102
Fenvalerate	82	93	79	111	118	85	99	109	81
Fipronil	89	110	92	119	119	96	74	104	83
Fludioxonil	<LOD	<LOD	55	104	117	68	87	117	98
Fluquinconazole	99	102	82	96	108	84	92	110	91
Flusilazole	90	119	99	123	112	85	75	99	101
Flutolanil	88	116	100	93	114	86	87	122	99
Flutriafol	85	108	91	77	114	66	81	114	92
Fluvalinate	35	97	77	121	122	91	98	101	76
Folpet**	133	34	45	66	36	29	<LOD	<LOD	<LOQ

Table 4 continued

** spiking levels are 50, 100 & 500 ng/g ** spiking levels are 100, 200 & 1000 ng/g <LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry			Wheat Flour			Leek		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
HCH alpha	79	109	87	113	121	88	84	135	108
HCH beta	85	111	90	109	110	85	87	138	109
HCH gamma_Lindane	87	110	89	115	123	88	81	132	106
Hexaconazole*	95	97	84	95	103	75	90	111	88
Imazalil*	72	97	84	87	102	67	69	96	78
Iprodione	109	111	86	120	124	94	109	102	84
Isofenphos-methyl	85	111	92	99	112	89	94	128	103
Kresoxim-methyl	86	111	96	114	119	85	86	120	101
Linuron*	126	118	95	95	100	63	126	133	98
Malathion	108	106	90	83	122	101	150	121	88
Mepanipyrim	82	111	96	123	138	72	93	121	95
Metalaxyl*	84	111	91	97	115	90	75	115	95
Methacrifos	89	108	78	82	109	96	66	130	103
Methamidophos	56	60	63	59	61	50	97	73	51
Methidathion	110	106	84	99	118	94	106	125	98
Methiocarb**	85	98	81	<LOD	<LOD	75	<LOD	<LOQ	78
Metribuzin*	87	111	98	89	117	84	94	129	99
Monocrotophos*	90	92	74	110	99	60	107	87	63
Myclobutanil	91	115	96	104	109	83	77	116	94
Ortho-phenylphenol	95	102	74	63	75	78	61	120	99
Oxadiazon	84	115	95	111	117	81	69	117	100
Oxadixyl	89	108	87	116	118	84	76	108	93
Paclobutrazol	81	106	91	95	109	85	90	111	91
Paraoxon-methyl*	102	108	109	137	146	111	132	117	73
Parathion (ethyl)*	69	98	101	54	95	95	120	132	100
Parathion-methyl	83	107	98	108	129	95	101	138	105
Pendimethalin	45	81	118	51	73	85	117	132	96
Permethrin	109	107	83	109	115	81	91	112	94
Phenthoate*	83	111	105	124	124	95	99	125	97
Phosalone	115	106	82	97	87	83	103	108	86
Phosmet	114	87	71	104	115	88	107	85	63
Phosphamidon	109	112	95	115	131	98	53	64	120
Pirimicarb	85	110	87	90	113	90	77	118	94
Pirimicarb-p-desmetyl*	79	99	81	85	106	82	82	122	89
Pirimiphos methyl	90	109	93	116	113	93	71	111	92
Prochloraz*	117	94	72	112	124	87	76	86	71
Procymidone	85	107	87	84	115	86	82	119	98
Profenofos	112	107	89	112	108	90	119	89	77
Propargite	104	104	90	<LOD	<LOD	58	62	89	88
Propiconazole	89	95	74	102	110	77	79	107	90
Propyzamide	84	110	89	100	116	88	90	133	103
Prothiofos	73	96	92	95	99	82	89	104	81
Pyraclostrobin	100	116	90	128	139	97	101	123	92
Pyridaben	111	110	86	108	114	81	92	108	87
Pyrimethanil	78	103	84	84	103	84	70	118	95

Table 4 continued ** spiking levels are 50, 100 & 500 ng/g ** spiking levels are 100, 200 & 1000 ng/g <LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry			Wheat Flour			Leek		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
Pyriproxyfen	101	107	84	106	113	81	90	112	92
Quinoxyfen	89	97	79	95	100	75	81	105	85
Spirodiclofen	91	88	78	132	113	75	<LOQ	96	71
Tebuconazole	80	95	75	100	111	79	89	110	91
Tebufenocide*	86	101	43	<LOD	<LOQ	84	<LOQ	106	87
Tebufenpyrad	80	102	89	101	104	89	70	91	78
Tefluthrin	85	109	87	86	109	86	72	126	102
Tetraconazole*	84	108	93	98	115	89	79	118	101
Tetradifon	77	119	106	104	112	78	75	116	95
Tetrahydrophthalimide (THPI)	<LOQ	<LOQ	90	<LOQ	117	115	<LOQ	111	95
Thiabendazole*	77	96	82	83	88	67	75	97	79
Tolclofos-methyl	81	108	89	102	110	84	91	119	83
Tolyfluanid*	111	71	67	87	79	77	n.d.	n.d.	n.d.
Triadimefon*	76	106	98	95	111	88	91	121	100
Triadimenol*	79	106	87	96	110	82	77	103	90
Trifloxystrobin	103	111	87	103	112	87	94	123	97
Trifluralin	121	84	59	54	39	50	77	50	87
Triticonazole	101	105	82	106	112	81	88	106	86
Vinclozolin	89	114	94	130	107	75	67	111	90

Table 5. Method precision and intermediate precision values [RSD %] at 10 ng/g (level 1), 20 ng/g (level 2) and 100 ng/g (level3). ** spiking levels are 50, 100 & 500 ** spiking levels are 100, 200 & 1000 <LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry				Wheat Flour				Leek			
	Precision			Intermediate Precision	Precision			Intermediate Precision	Precision			Intermediate Precision
	Level 1	Level 2	Level 3		Level 1	Level 2	Level 3		Level 1	Level 2	Level 3	
Acephate	7	7	12	15	29	4	27	9	12	18	8	22
Acrinathrin	32	51	18	37	9	3	7	5	4	20	17	22
Amitraz**	5	11	11	14	n.d.	n.d.	n.d.	n.d.	3	7	15	27
Azinphos-methyl*	2	4	7	5	6	1	6	3	6	10	7	23
Azoxystrobin*	3	6	2	7	12	4	4	12	5	6	10	11
Bifenthrin	6	10	3	9	13	6	5	9	15	12	8	13
Bitertanol	2	3	2	4	4	2	4	2	2	6	11	10
Boscalid (Nicobifen)	3	3	2	3	3	2	3	2	6	2	11	7
Bromopropylate	7	8	4	10	10	5	6	8	5	13	7	13
Bromuconazole	2	4	2	4	7	3	4	3	2	6	9	11
Bupirimate*	6	3	3	4	3	3	3	3	6	5	9	6
Buprofezin*	6	4	3	5	4	3	3	4	2	4	8	4
Cadusafos	7	9	3	8	15	3	12	5	2	7	6	6
Captan	31	64	15	75	28	21	52	66	n.d.	n.d.	n.d.	n.d.
Carbaryl*	10	13	8	25	18	3	20	9	<LOQ	22	15	29
Carbofuran	18	5	4	16	27	5	17	11	11	40	20	50
Carboxin	7	4	2	7	6	4	3	6	4	5	7	7

Table 5 continued

** spiking levels are 50, 100 & 500 ** spiking levels are 100, 200 & 1000 <LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry				Wheat Flour				Leek			
	Precision			Intermediate Precision	Precision			Intermediate Precision	Precision			Intermediate Precision
	Level 1	Level 2	Level 3		Level 1	Level 2	Level 3		Level 1	Level 2	Level 3	
Chlorfenapyr*	5	7	5	6	11	5	6	6	4	5	6	8
Chlorfenvinphos	4	5	4	7	33	5	15	37	3	4	8	10
Chlorobenzilate	3	7	4	6	5	3	38	4	3	5	8	6
Chlorothalonil	4	18	18	16	38	9	27	11	n.d.	n.d.	n.d.	n.d.
Chlorpropham	4	5	3	4	20	4	12	6	4	8	5	5
Chlorpyrifos-ethyl	5	7	2	6	11	7	10	8	13	7	7	6
Chlorpyrifos-methyl	5	3	5	6	17	3	12	6	8	5	6	5
Cyfluthrin	6	7	3	7	4	2	5	3	9	19	13	19
Cyhalothrin	8	25	9	19	3	3	3	3	3	20	15	18
Cypermethrin	6	12	5	9	11	2	3	3	17	9	12	13
Cyproconazole	5	4	3	4	4	2	2	3	5	6	7	4
Cyprodinil	3	8	3	6	7	5	8	7	8	6	6	8
DDD p,p	2	3	2	5	2	4	3	4	4	5	6	7
DDE p, p	7	7	4	6	6	4	2	5	4	6	5	16
DDT p,p	4	9	4	18	10	3	7	6	3	4	9	9
Deltamethrin	9	32	11	23	15	2	4	6	5	18	13	19
Demeton-S-methyl*	1	6	4	5	12	4	13	9	5	4	7	11
Diazinon	7	9	3	6	14	4	13	6	12	8	6	6
Dichlofluanid*	8	25	12	20	56	20	17	17	<LOD	<LOD	90	n.d.
Dichloran	11	10	4	8	11	5	16	6	10	8	5	8
Dichlorbenzophenon, p,p'-	14	18	5	14	<LOD	<LOQ	11	n.d.	<LOQ	16	7	18
Dichlorvos	5	7	8	13	28	5	19	9	8	7	10	9
Dicofol	9	4	2	5	5	1	4	4	5	2	10	8
Difenoconazole	7	4	3	10	19	5	5	14	10	5	3	12
Dimethoate	10	10	6	12	17	3	11	10	5	5	6	8
Dimethomorph	5	3	3	11	18	7	9	13	5	7	9	6
Diphenylamine	7	7	3	6	33	12	19	21	8	12	7	8
Endosulfan*	9	10	5	8	6	7	46	21	17	8	6	6
Endosulfan sulfate	9	3	4	5	4	3	4	3	4	6	9	6
EPN	4	3	3	11	8	4	4	11	4	6	9	6
Epoxiconazole	4	5	2	4	6	3	6	2	5	4	7	5
Ethion	1	3	2	3	4	2	3	2	8	7	9	5
Ethoprop (Ethoprophos)	2	7	2	5	16	4	13	12	4	6	6	5
Etofenprox	3	5	2	6	4	2	2	2	3	3	9	6
Fenamiphos	9	7	3	8	10	4	7	6	5	9	12	10
Fenamiphos sulfone	11	30	10	27	12	2	5	11	15	19	17	16
Fenamiphos-sulfoxid**	8	22	7	28	26	3	12	17	9	5	21	9
Fenarimol	3	3	1	3	7	2	3	3	3	5	9	9
Fenbuconazol	3	5	3	4	3	3	3	2	1	6	10	5
Fenitrothion	9	7	4	8	16	4	11	8	5	5	7	7
Fenoxycarb	3	3	2	3	6	5	5	7	2	7	8	8
Fenpropathrin	9	4	2	4	<LOD	<LOD	8	n.d.	<LOD	<LOD	13	n.d.
Fenpropidin*	27	26	11	21	29	12	61	15	n.d.	37	17	42

Table 5 continued

** spiking levels are 50, 100 & 500 ** spiking levels are 100, 200 & 1000 <LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry				Wheat Flour				Leek			
	Precision			Intermediate Precision	Precision			Intermediate Precision	Precision			Intermediate Precision
	Level 1	Level 2	Level 3		Level 1	Level 2	Level 3		Level 1	Level 2	Level 3	
Fenpropimorph	7	11	3	7	14	6	8	8	18	10	9	9
Fenthion	16	5	6	13	30	12	9	48	12	13	5	10
Fenvalerate	5	8	3	7	7	2	3	2	3	4	12	9
Fipronil	8	5	3	7	24	4	18	17	11	4	4	17
Fludioxonil	<LOD	<LOD	6	n.d.	7	5	46	5	6	9	5	8
Fluquinconazole	7	7	5	7	13	4	4	11	3	6	10	16
Flusilazole	12	14	3	9	7	7	4	7	14	15	6	15
Flutolanil	5	2	2	5	8	4	6	5	4	8	8	8
Flutriafol	6	1	2	5	23	2	40	5	6	6	7	8
Fluvalinate	8	12	5	9	6	5	4	4	9	14	14	14
Folpet**	30	71	21	74	27	12	43	22	<LOD	<LOD	<LOQ	n.d.
HCH alpha	7	9	3	6	9	4	11	5	3	8	5	13
HCH beta	7	8	3	7	18	4	10	7	7	8	7	12
HCH gamma_Lindane	10	9	1	7	12	4	11	4	10	9	6	10
Hexaconazole*	14	12	3	9	11	6	4	7	13	4	10	8
Imazalil*	7	4	3	4	9	5	10	4	6	4	10	8
Iprodione	12	4	4	5	6	5	8	7	7	7	11	10
Isofenphos-methyl	3	3	3	3	13	4	9	6	4	4	7	7
Kresoxim-methyl	1	6	4	5	5	2	5	3	8	4	6	5
Linuron*	5	5	7	18	18	6	20	12	5	4	10	9
Malathion	3	8	4	8	14	4	14	10	10	11	10	12
Mepanipyrim	10	4	3	6	13	4	22	8	4	7	9	5
Metalaxyl*	5	5	5	5	17	5	12	6	2	7	4	6
Methacrifos	11	11	3	7	75	3	17	8	7	11	6	11
Methamidophos	15	12	14	31	23	7	25	8	7	14	9	22
Methidathion	6	7	5	7	14	3	13	5	5	4	8	6
Methiocarb**	15	15	5	17	<LOD	<LOD	11*	n.d.	<LOD	<LOQ	11	n.d.
Metribuzin*	8	7	6	9	15	5	11	6	5	7	7	7
Monocrotophos*	13	13	11	20	9	2	13	6	6	15	10	18
Myclobutanil	5	3	3	6	1	5	3	4	6	9	5	7
Ortho-phenylphenol	4	7	3	6	31	12	15	20	4	8	6	6
Oxadiazon	3	9	4	7	5	4	6	4	9	8	6	8
Oxadixyl	4	4	4	5	8	2	4	3	7	5	8	8
Paclobutrazol	3	6	5	4	16	4	10	7	4	4	8	6
Paraoxon-methyl*	7	10	12	17	12	4	17	10	4	16	16	16
Parathion (ethyl)*	8	10	3	12	25	8	11	17	4	5	7	5
Parathion-methyl	13	11	6	9	8	7	12	8	4	5	7	4
Pendimethalin	22	20	5	25	17	13	11	32	8	9	6	14
Permethrin	3	4	3	4	2	3	4	3	4	10	11	8
Phenthoate*	5	7	4	6	5	3	13	23	3	5	8	4
Phosalone	2	5	2	4	42	3	3	22	4	10	8	10
Phosmet	7	10	9	14	6	1	8	4	6	23	13	26
Phosphamidon	7	9	10	21	20	2	15	8	76	42	24	62

Table 5 continued

** spiking levels are 50, 100 & 500 ** spiking levels are 100, 200 & 1000 <LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry				Wheat Flour				Leek			
	Precision			Intermediate Precision	Precision			Intermediate Precision	Precision			Intermediate Precision
	Level 1	Level 2	Level 3		Level 1	Level 2	Level 3		Level 1	Level 2	Level 3	
Pirimicarb	9	7	5	6	13	5	12	6	7	9	6	7
Pirimicarb-p-desmetyl*	12	16	8	11	13	3	12	5	4	8	9	5
Pirimiphos methyl	6	6	4	5	10	4	11	22	6	7	6	5
Prochloraz*	4	3	4	7	9	5	7	10	5	6	14	6
Procymidone	8	6	4	7	16	2	8	10	8	6	7	9
Profenofos	19	12	7	13	14	8	12	12	13	27	12	31
Propargite	5	4	4	11	<LOD	<LOD	56	n.d.	10	9	8	16
Propiconazole	2	2	3	4	8	4	11	3	3	6	8	6
Propyzamide	4	6	3	5	11	3	11	5	2	7	5	5
Prothiofos	9	3	4	6	17	5	8	17	8	10	6	11
Pyraclostrobin	5	3	3	8	2	2	3	2	2	3	11	5
Pyridaben	3	2	3	4	3	2	2	3	6	8	12	9
Pyrimethanil	4	7	2	6	13	3	8	5	3	9	7	6
Pyriproxyfen	3	4	2	4	4	1	3	1	2	4	8	6
Quinoxyfen	2	4	2	3	4	2	2	5	5	3	8	3
Spirodiclofen	10	6	5	13	14	5	10	10	<LOQ	11	12	17
Tebuconazole	3	2	1	3	6	3	7	2	3	3	9	7
Tebufenocide*	6	5	2	6	<LOD	<LOQ	6	7	<LOQ	6	9	7
Tebufenpyrad	3	6	3	5	18	7	10	16	3	8	8	6
Tefluthrin	7	7	4	5	16	5	11	6	4	8	6	6
Tetraconazole*	4	6	4	4	13	4	11	6	4	4	6	4
Tetradifon	11	7	4	5	7	2	6	4	7	6	7	7
Tetrahydrophthalimide (THPI)	<LOQ	<LOQ	8	n.d.	<LOQ	27	11	23	<LOQ	9	6	8
Thiabendazole*	5	3	4	3	16	3	14	6	4	5	9	7
Tolclofos-methyl	4	6	6	9	15	6	15	18	5	8	5	20
Tolyfluanid*	9	22	10	20	21	8	17	10	n.d.	n.d.	n.d.	n.d.
Triadimefon*	4	4	2	5	12	3	9	6	1	6	6	7
Triadimenol*	9	7	3	7	10	3	9	10	9	7	7	5
Trifloxystrobin	4	4	2	3	6	4	3	3	4	8	8	6
Trifluralin	3	16	8	17	1	12	13	20	2	12	8	32
Triticonazole	7	3	3	3	4	3	2	3	5	6	9	6
Vinclozolin	15	4	6	8	16	6	14	14	23	9	7	10

Table 6: Method LOD, LOQ and current legislative residue level values *(all values in ng/g).* ** default value of 10 ng/g set as no MRL values defined*

Compound	Strawberry			Wheat Flour			Leek		
	LOD	LOQ	MRL	LOD	LOQ	MRL	LOD	LOQ	MRL
Acephate	0.3	1	20	0.6	2	20	1.5	5	20
Acrinathrin	6	20	200	2.7	9	50	6	20	50
Amitraz	6	20	50	300	1000	50	12	40	50
Azinphos-methyl	3	10	50	0.9	3	50	2.4	8	50
Azoxystrobin	0.9	3	1000	0.3	1	300	1.5	5	1000
Bifenthrin	6	20	500	4.8	16	500	7.5	25	50
Bitertanol	0.9	3	50	0.6	2	50	0.6	2	50
Boscalid (Nicobifen)	0.3	1	1000	0.15	0.5	500	0.6	2	5000
Bromopropylate	3	10	10	2.1	7	10	1.5	5	10
Bromuconazole	2.4	8	50	0.27	0.9	200	1.2	4	50
Bupirimate	3	10	1000	3	10	50	4.5	15	50
Buprofezin	6	20	3000	15	50	50	4.5	15	50
Cadusafos	1.5	5	10	0.3	1	10	1.5	5	10
Captan	3	10	3000	3	10	20	1000	1500	2000
Carbaryl	4.5	15	50	4.5	15	500	4.5	15	50
Carbofuran	9	30	20	3	10	20	4.5	15	20
Carboxin	1.8	6	20	6	20	20	0.6	2	20
Chlorfenapyr	4.5	15	10*	1.5	5	10*	3	10	10*
Chlorfenvinphos	1.5	5	10*	0.3	1	10*	1.2	4	10*
Chlorobenzilate	0.9	3	20	0.3	1	20	1.2	4	20
Chlorothalonil	12	40	5000	0.3	1	100	1500	2500	40000
Chlorpropham	1.5	5	50	0.6	2	20	1.2	4	50
Chlorpyrifos-ethyl	1.5	5	10*	0.3	1	10*	1.5	5	10*
Chlorpyrifos-methyl	1.5	5	500	0.3	1	3000	0.75	2.5	50
Cyfluthrin	4.5	15	20	3.6	12	20	2.4	8	20
Cyhalothrin	1.8	6	10*	0.9	3	10*	1.5	5	10*
Cypermethrin	4.5	15	70	15	50	2000	4.5	15	500
Cyproconazole	1.5	5	50	1.8	6	100	1.5	5	50
Cyprodinil	1.2	4	5000	0.3	1	500	1.5	5	50
DDD p,p	0.3	1	50	0.21	0.7	50	0.75	2.5	50
DDE p,p	0.3	1	10*	0.24	0.8	10*	1.2	4	10*
DDT o,p	0.6	2	10*	0.9	3	10*	0.6	2	10*
Deltamethrin	4.5	15	200	2.4	8	2000	7.5	25	200
Demeton-S-methyl	1.5	5	10*	1.5	5	10*	1.2	4	10*
Diazinon	0.3	1	10	0.3	1	20	0.3	1	10
Dichlofluanid	13.5	45	10*	3	10	10*	150	500	10*
Dichloran	4.5	15	300	3	10	10	2.4	8	100
Dichlorbenzophenon, p,p'-	3	10	10*	15	50	10*	4.5	15	10*
Dichlorvos	3	10	10	3	10	10	2.7	9	10
Dicofol	2.4	8	20	1.5	5	20	1.5	5	20
Difenoconazole	1.5	5	400	1.2	4	100	0.9	3	500
Dimethoate	1.2	4	20	0.6	2	50	0.6	2	20
Dimethomorph	1.5	5	10*	1.5	5	10*	0.6	2	10*

Table 6 continued

** default value of 10 ng/g set as no MRL values defined*

Compound	Strawberry			Wheat Flour			Leek		
	LOD	LOQ	MRL	LOD	LOQ	MRL	LOD	LOQ	MRL
Diphenylamine	0.3	1	50	0.3	1	50	0.6	2	50
Endosulfan	1.5	5	50	6	20	50	1.2	4	50
Endosulfan sulfate	0.6	2	10*	1.5	5	10*	0.9	3	10*
EPN	2.1	7	10*	2.1	7	10*	0.9	3	10*
Epoxiconazole	1.2	4	50	0.6	2	600	0.6	2	50
Ethion	1.2	4	10	0.9	3	10	0.6	2	10
Ethoprop (Ethoprophos)	0.3	1	20	0.6	2	20	0.9	3	20
Etofenprox	0.9	3	1000	0.9	3	500	0.6	2	10
Fenamiphos	1.2	4	20	1.2	4	20	0.9	3	20
Fenamiphos sulfone	3.6	12	10*	0.9	3	10*	1.5	5	10*
Fenamiphos-sulfoxid	0.9	3	10*	10.5	35	10*	7.5	25	10*
Fenarimol	0.9	3	300	0.3	1	20	0.3	1	20
Fenbuconazol	0.6	2	50	0.6	2	100	0.75	2.5	50
Fenitrothion	3	10	10	1.5	5	50	2.4	8	10
Fenoxycarb	0.9	3	50	1.2	4	50	0.75	2.5	50
Fenpropathrin	7.5	25	2000	30	100	10	30	100	10
Fenpropidin	4.5	15	50	12	40	500	7.8	26	50
Fenpropimorph	0.15	0.5	1000	0.3	1	500	1.2	4	1000
Fenthion	1.5	5	10	1.8	6	10	1.5	5	10
Fenvalerate	2.25	7.5	20	1.5	5	50	0.9	3	20
Fipronil	0.3	1	5	1.5	5	5	0.9	3	10
Fludioxonil	30	100	3000	1.2	4	200	1.2	4	50
Fluquinconazole	0.6	2	50	0.3	1	100	0.6	2	50
Flusilazole	4.5	15	20	2.4	8	100	1.5	5	20
Flutolanil	0.6	2	50	0.3	1	50	0.6	2	50
Flutriafol	0.3	1	500	0.9	3	500	0.45	1.5	50
Fluvalinate	6	20	10*	3.6	12	10*	4.5	15	10*
Folpet	75	250	3000	450	1500	2000	600	2000	20
HCH alpha	0.3	1	10	0.3	1	20	0.3	1	10
HCH beta	0.3	1	10	0.3	1	20	0.3	1	10
HCH gamma_Lindane	0.15	0.5	10	0.3	1	10	0.6	2	10
Hexaconazole	9	0	200	4.5	15	100	4.5	15	20
Imazalil	1.5	0	50	6	20	50	1.8	6	50
Iprodione	1.5	5	1000	1.5	5	500	1.2	4	20
Isofenphos-methyl	0.3	1	10*	0.3	1	10*	1.2	4	10*
Kresoxim-methyl	1.5	5	1000	1.8	6	50	1.5	5	5000
Linuron	3	10	50	1.8	6	50	1.5	5	50
Malathion	3	10	20	10.5	35	8000	3.6	12	20
Mepanipyrim	1.8	6	2000	2.4	8	10	1.2	4	10
Metalaxyl	9	30	500	10.5	35	50	7.5	25	200
Methacrifos	0.9	3	50	1.8	6	50	0.9	3	50
Methamidophos	0.75	2.5	10	0.9	3	10	1.5	5	10
Methidathion	0.6	2	20	0.9	3	20	1.5	5	20

Table 6 continued

** default value of 10 ng/g set as no MRL values defined*

Compound	Strawberry			Wheat Flour			Leek		
	LOD	LOQ	MRL	LOD	LOQ	MRL	LOD	LOQ	MRL
Methiocarb	150	500	1000	300	1000	100	135	450	200
Metribuzin	0.6	2	100	1.8	6	100	2.1	7	100
Monocrotophos	3	10	10*	4.5	15	10*	3	10	10*
Myclobutanil	0.3	1	1000	1.2	4	20	1.2	4	20
Ortho-phenylphenol	1.5	5	10*	1.5	5	10*	1.5	5	10*
Oxadiazon	0.3	1	50	0.9	3	50	0.6	2	50
Oxadixyl	3	10	10	5.4	18	10	3	10	70
Paclobutrazol	0.9	3	500	0.3	1	20	1.2	4	20
Paraoxon-methyl	6	20	20	6	20	20	3	10	20
Parathion (ethyl)	12	40	10*	37.5	125	10*	12	40	10*
Parathion-methyl	0.6	2	10*	1.2	4	10*	1.5	5	10*
Pendimethalin	1.5	5	50	1.2	4	50	2.1	7	50
Permethrin	2.4	8	50	1.8	6	50	4.5	15	50
Phenthoate	12	40	10*	1.8	6	10*	7.5	25	10*
Phosalone	1.8	6	50	1.2	4	50	1.5	5	50
Phosmet	0.24	0.8	50	0.3	1	50	0.6	2	50
Phosphamidon	0.3	1	10	3	10	10	3	10	10
Pirimicarb	0.9	3	3000	0.9	3	500	0.6	2	1000
Pirimicarb-p-desmetyl	0.9	3	10*	1.2	4	10*	1.5	5	10*
Pirimiphos methyl	0.27	0.9	50	0.6	2	5000	3	10	50
Prochloraz	15.6	52	50	30	100	500	15	50	50
Procymidone	3	10	20	3.9	13	20	1.8	6	20
Profenofos	3	10	50	2.1	7	50	2.1	7	50
Propargite	3	10	10	30	100	10	7.5	25	10
Propiconazole	1.8	6	50	1.2	4	50	0.6	2	100
Propyzamide	0.21	0.7	20	0.9	3	20	0.6	2	20
Prothiofos	2.4	8	10*	0.9	3	10*	1.5	5	10*
Pyraclostrobin	0.75	2.5	1000	0.3	1	100	0.3	1	500
Pyridaben	0.9	3	1000	1.8	6	50	1.5	5	50
Pyrimethanil	0.9	3	5000	1.5	5	50	1.2	4	1000
Pyriproxyfen	0.3	1	50	1.2	4	50	0.6	2	50
Quinoxyfen	0.15	0.5	300	0.24	0.8	20	0.6	2	20
Spirodiclofen	6	20	2000	6	20	20	6	20	20
Tebuconazole	1.5	5	50	0.24	0.8	200	0.3	1	1000
Tebufenocide	30	100	50	60	200	50	30	100	50
Tebufenpyrad	0.3	1	500	0.6	2	50	0.6	2	50
Tefluthrin	0.15	0.5	50	0.3	1	50	1.5	5	50
Tetraconazole	2.4	8	200	1.5	5	100	1.2	4	20
Tetradifon	1.2	4	10	1.8	6	10	0.9	3	10
Tetrahydrophthalimide (THPI)	7.5	25	10*	4.5	15	10*	4.5	15	10*
Thiabendazole	4.5	15	50	1.5	5	50	2.7	9	50
Tolclofos-methyl	0.3	1	50	0.6	2	50	2.1	7	50
Tolyfluanid	7.5	25	5000	1.8	6	50	1000	3000	10*

Table 6 continued

** default value of 10 ng/g set as no MRL values defined*

Compound	Strawberry			Wheat Flour			Leek		
	LOD	LOQ	MRL	LOD	LOQ	MRL	LOD	LOQ	MRL
Triadimefon	0.6	2	500	2.1	7	200	1.2	4	100
Triadimenol	7.5	25	500	2.1	7	200	2.7	9	100
Trifloxystrobin	1.5	5	500	1.2	4	50	1.2	4	200
Trifluralin	15	50	100	4.5	15	100	3	10	500
Triticonazole	1.5	5	10	0.6	2	10	1.5	5	10
Vinclozolin	2.4	8	50	0.9	3	50	2.7	9	50

Table 7: External quality control (FAPAS) results for the relevant compounds.

Compound	Fapas Sample Number	Assigned Value [µg/kg]	Acceptance Range [µg/kg]	Measured Value [µg/kg] (RSD%)
Carbaryl	T19142	89	49.9-128.2	51.2 (22)
beta Endosulfan	T19140	93.6	52.4-134.9	91.3 (7)
Chlorpyrifos-methyl	T19141	86.0	48.2-123.9	88.8 (8)
Cypermethrin	T19141	128.8	72.3-184.1	111.9 (8)
Cypermethrin	T19142	140.4	80.0-200.7	120.2 (17)
DDT, o,p	T19141	67.4	37.8-97.1	38.7 (16)
Dicloran	T19142	66.3	37.1-95.5	63.1 (15)
Dimethoate	T19141	69.0	38.6-99.4	62.3 (15)
Ethoprophos	T19142	29.3	16.4-42.4	25.7 (10)
Methidathion	T19141	29.0	16.3-41.8	29.1 (19)
Monocrotophos	T19141	26.4	14.8-38.0	36.8 (13)
Phosalone	T19140	70.4	39.4-101.4	68.3 (9)
Propyzamide	T19140	89.9	50.4-129.5	94.7 (4)

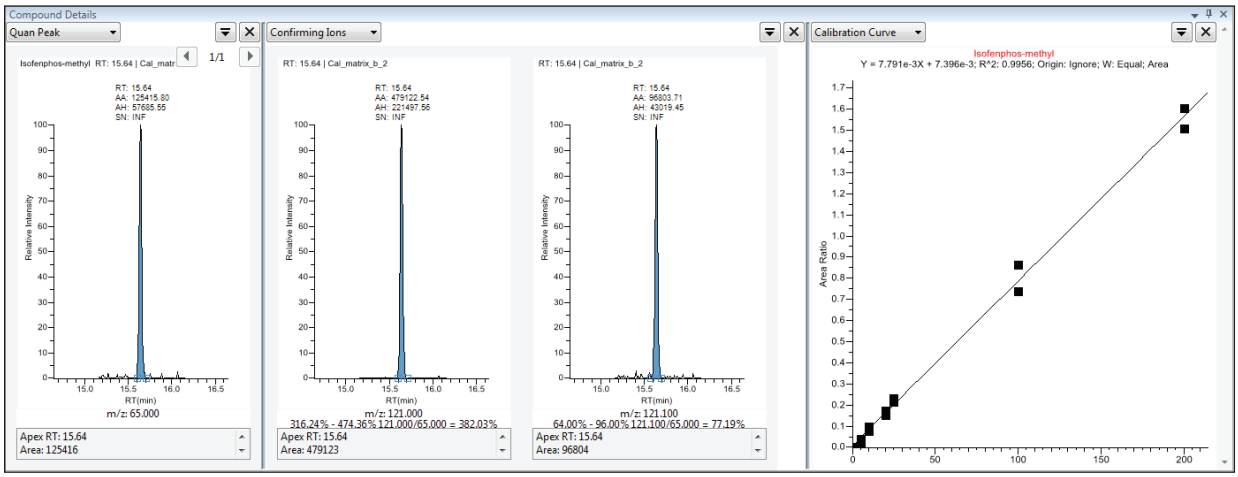

Figure 1. Chromatogram of isofenphos-methyl in leek at at calibration level 2 [5ng/g].

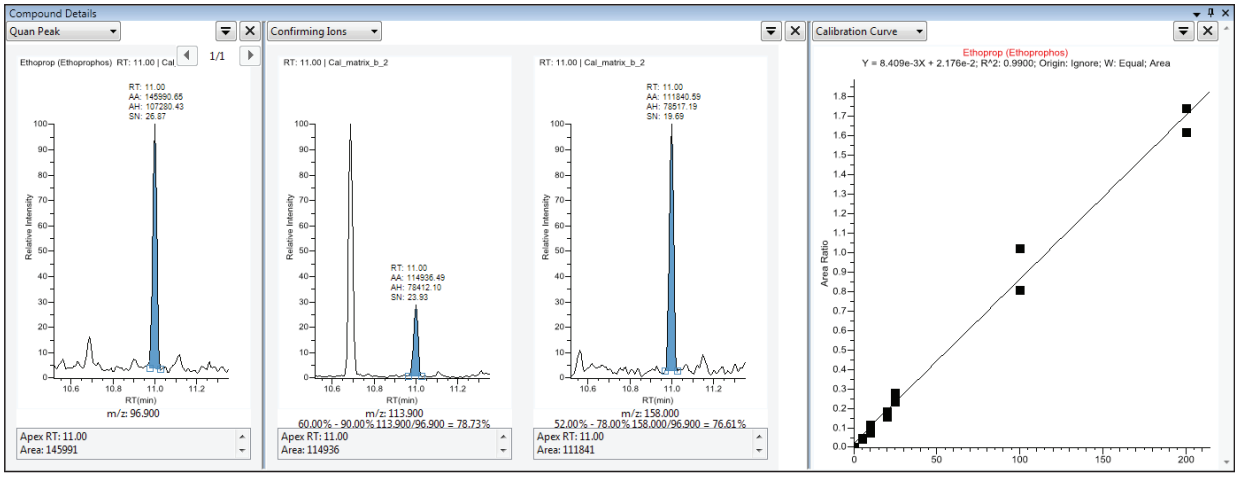

Figure 2. Chromatogram of ethoprop in leek at calibration level2 [5ng/g].

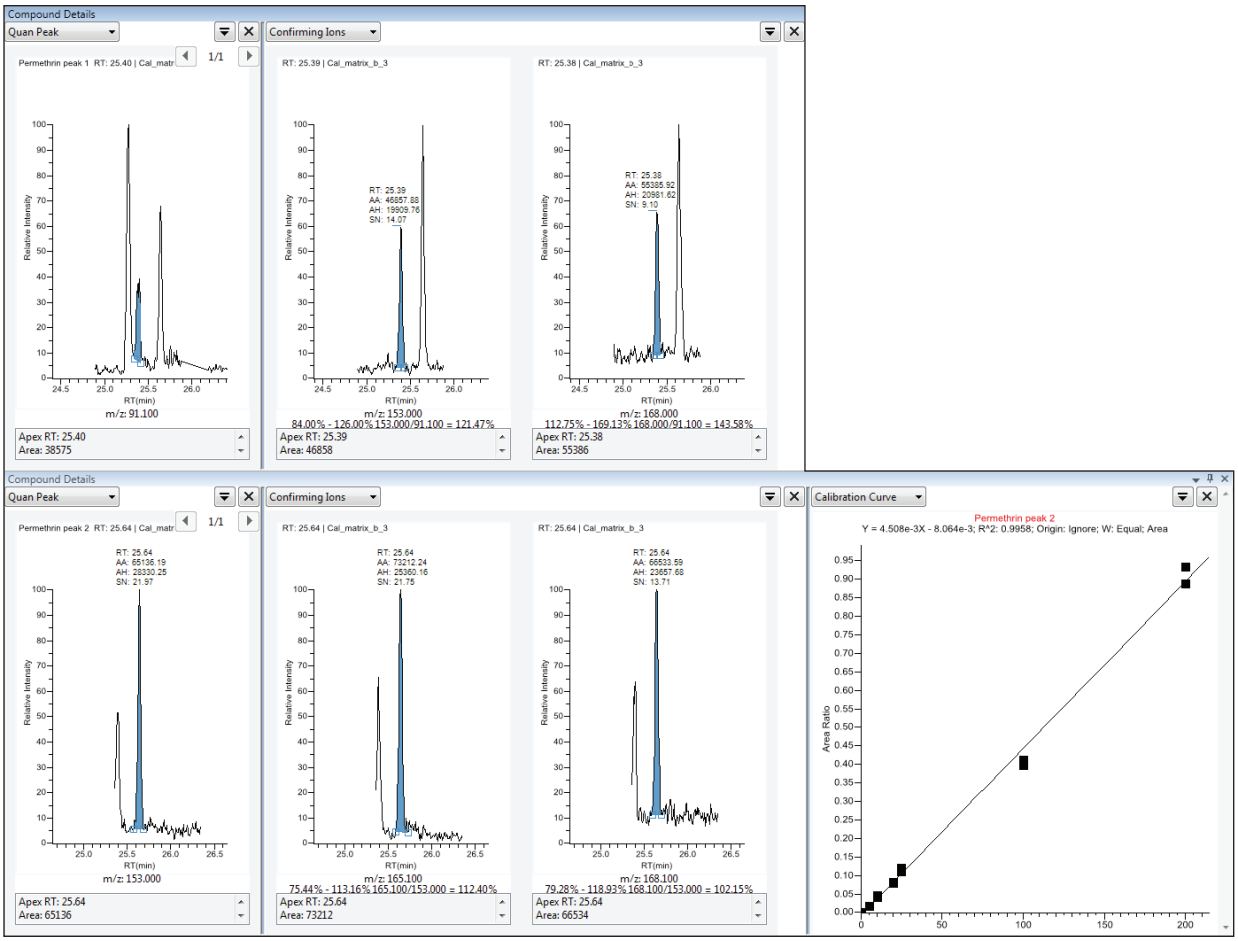

Figure 3. Chromatogram of both permethrine peaks in leek at calibration level3 [10ng/g].

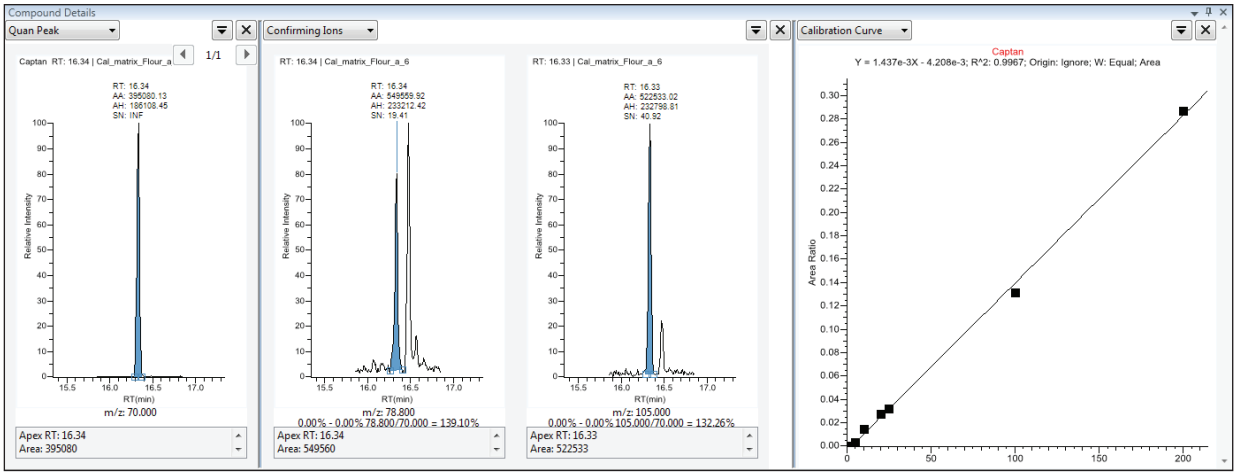

Figure 4. Chromatogram of captan in wheat flour at calibration level6 [100 ng/g].

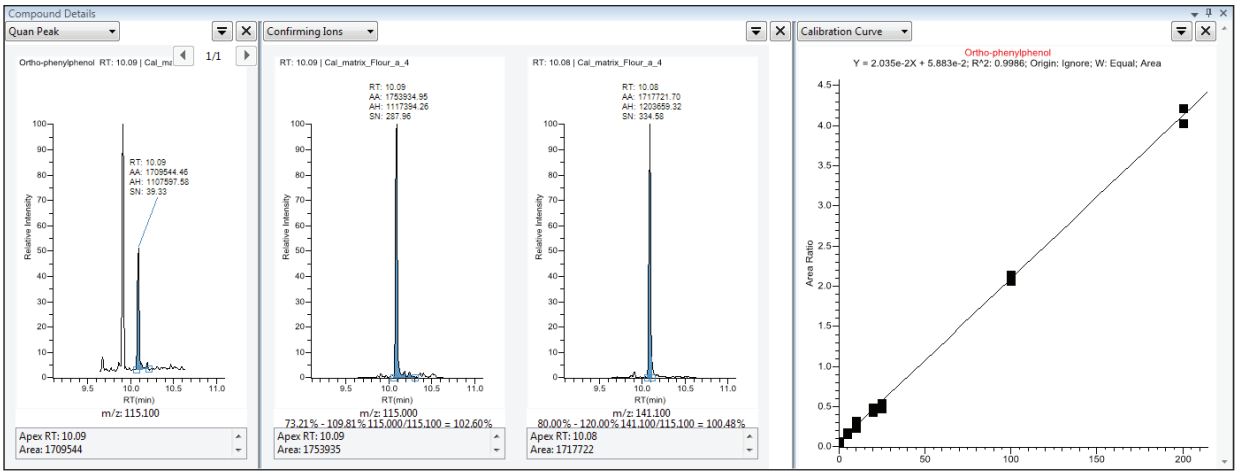

Figure 5. Chromatogram of o-phenylphenol wheat flour at calibration level4 [25ng/g].

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Technical Note: 10295

Multi-residue Pesticide Analysis in Green Tea by a Modified QuEChERS Extraction and Ion Trap GC/MS^n Analysis

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Key Words

- ITQ 700
- Food Safety
- GC/MS^n
- Green Tea
- Pesticide Residues
- QuEChERS

Introduction

Recently formulated pesticides are quite different in their physical properties from their predecessors such as 4,4'-DDT. Most of these newer pesticides are smaller in molecular weight and were designed to break down rapidly in the environment. Therefore, to successfully identify and quantify these compounds in foods, more careful consideration must be placed on the sample preparation for extraction and the instrument parameters for analysis. This study will cover the preparation of extracts and the optimization of the analytical parameters of the splitless injection, separation, and detection.

The determination of pesticides in fruits, vegetables, grains and herbs has been simplified by a new sample preparation method, QuEChERS (**Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe), published recently as AOAC Method 2007.01.[1] The sample preparation is simplified by using a single step buffered acetonitrile (MeCN) extraction and liquid-liquid partitioning from water in the sample by salting out with sodium acetate and magnesium sulfate ($MgSO_4$).[1] QuEChERS can be used to prepare green tea samples for analysis by gas chromatography/tandem mass spectrometry (GC/MS^n) on the Thermo Scientific ITQ 700 GC-ion trap mass spectrometer.

The study was performed to determine the linear ranges, quantitation limits and detection limits for a partial list of pesticides that are commonly used on green tea crops, prepared in matrix using the QuEChERS sample preparation guidelines. A splitless injection of 22 pesticides was made in a single injection with detection in electron ionization (EI) MS/MS. Since the extracts were prepared in MeCN, a solvent exchange was made to hexane/acetone (9:1) prior to conventional splitless injection.[2] Once the calibration curve was constructed, multiple matrix spikes were analyzed at levels of 37.5, 75, 150, 225, 600, or 1200 ng/g (ppb) and low level spikes of 7.5, 15, 37.5, 75, or 300 ng/g (ppb) to verify the precision and accuracy of the analytical method. These concentrations were chosen based on the requirements of various regulatory agencies.

Experimental Conditions

The sample preparation involves careful homogenization of the sample. Extraction solvents must be buffered and the powdered reagents measured at appropriate amounts for the size of sample prepared. Some reagents cause an exothermic reaction when mixed with water, which can adversely affect the recoveries of target compounds. The recommended consumables required for sample

preparation and analysis were rigorously tested (Table 1). A list of the pesticides to be studied was created that would address all of the various functional groups and different physical properties of most pesticides. MS^n parameters were optimized with the use of variable buffer gas, the testing of the isolation efficiency, and adjustment of the Collision Induced Dissociation (CID) voltage. A surge splitless injection was made into a Thermo Scientific TRACE TR-Pesticide III 35% diphenyl/65% dimethyl polysiloxane column, (0.25 mm x 30 meter, and a film thickness of 0.25 µm with a 5 m guard column).

Item Descriptions
TRACE™ TR-Pesticide III 35% diphenyl/65% dimethyl polysiloxane column, 0.25 mm x 30 meter, 0.25 µm w/ 5 m guard column
5 mm ID x 105 mm liner (pk of 5)
10 µL syringe
Septa (pk of 50)
Liner graphite seal (pk of 10)
Ion volume, EI open
Ion volume holder
Graphite ferrule 0.1-0.25 mm (pk of 10)
Ferrule 0.4 mm ID 1/16 G/V (pk of 10)
Blank vespel ferrule for MS interface (pk of 10)
2 mL amber glass vial, silanized glass, with write-on patch (pk of 100)
Blue cap with ivory PTFE/red rubber seal (pk of 100)
Acetonitrile analytical grade (4L)
Hexane GC Resolv* (4L)
Acetone GC Resolv* (4L)
Organic bottle top dispenser
HPLC grade glacial acetic acid
50 mL Nalgene FEP centrifuge tubes (pk of 2)
Clean up tube:15 mL tube ENVIRO 900 mg $MgSO_4$, 300 mg PSA 150 mg C18 (pk of 50)
50 mL PP Tubes 6 g $MgSO_4$, 1.5 g $CH_3CHOONa$ (anhydrous) (pk of 250)
Clean up tube: 2 mL tubes 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18 (pk of 100)

Table 1: Consumables for QuEChERS sample preparation and GC/MS analysis

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Sample Extraction and Clean Up

The QuEChERS sample preparation procedure consists of the steps shown in Figure 1. There are three parts: extraction, clean up, and solvent exchange. The solvent exchange provides a final solvent that is more amenable to splitless injection and concentrates the analytes to reach lower detection limits. In addition, the solvent exchange and final clean up removed caffeine and polyphenols from the sample before injection. These compounds readily dissolve in acetonitrile, as shown in Figure 2 (red trace). However, they are not readily soluble in hexane:acetone (9:1), as shown in the black trace in Figure 2. This helps keep the analytical system clean.

Care must be taken to adequately and thoroughly homogenize the sample. A large amount of water must be added during the homogenization step when preparing the tea for extraction. This must be taken into consideration in the final calculations of spikes and standards. A total of 1200 mL of water was added to 200 g of green tea in this experiment.

An observation was made during the extraction phase of the sample preparation. If the MeCN extract was poured into the $MgSO_4$, poor spike recoveries were observed. This was due to an exothermic reaction of any water in the sample and the $MgSO_4$. Although many vendors offer the pre-measured powder reagents in a separate capped centrifuge tube, it is recommended not to add the sample to these tubes. Instead, reagents from these tubes should instead be added directly to the sample containing the acidified MeCN. Therefore, an empty 50 mL FEP extraction tube was included in the list of consumables for sample preparation. A thoroughly homogenized 15 g sample of green tea and water were weighed into the FEP extraction tube. Then 15 mL of 1% glacial acetic acid MeCN extraction solvent was poured into the tube on top of the sample. The surrogate and the pesticide solutions were spiked into this MeCN layer for the method validation (MVD) and method detection limit (MDL) samples.

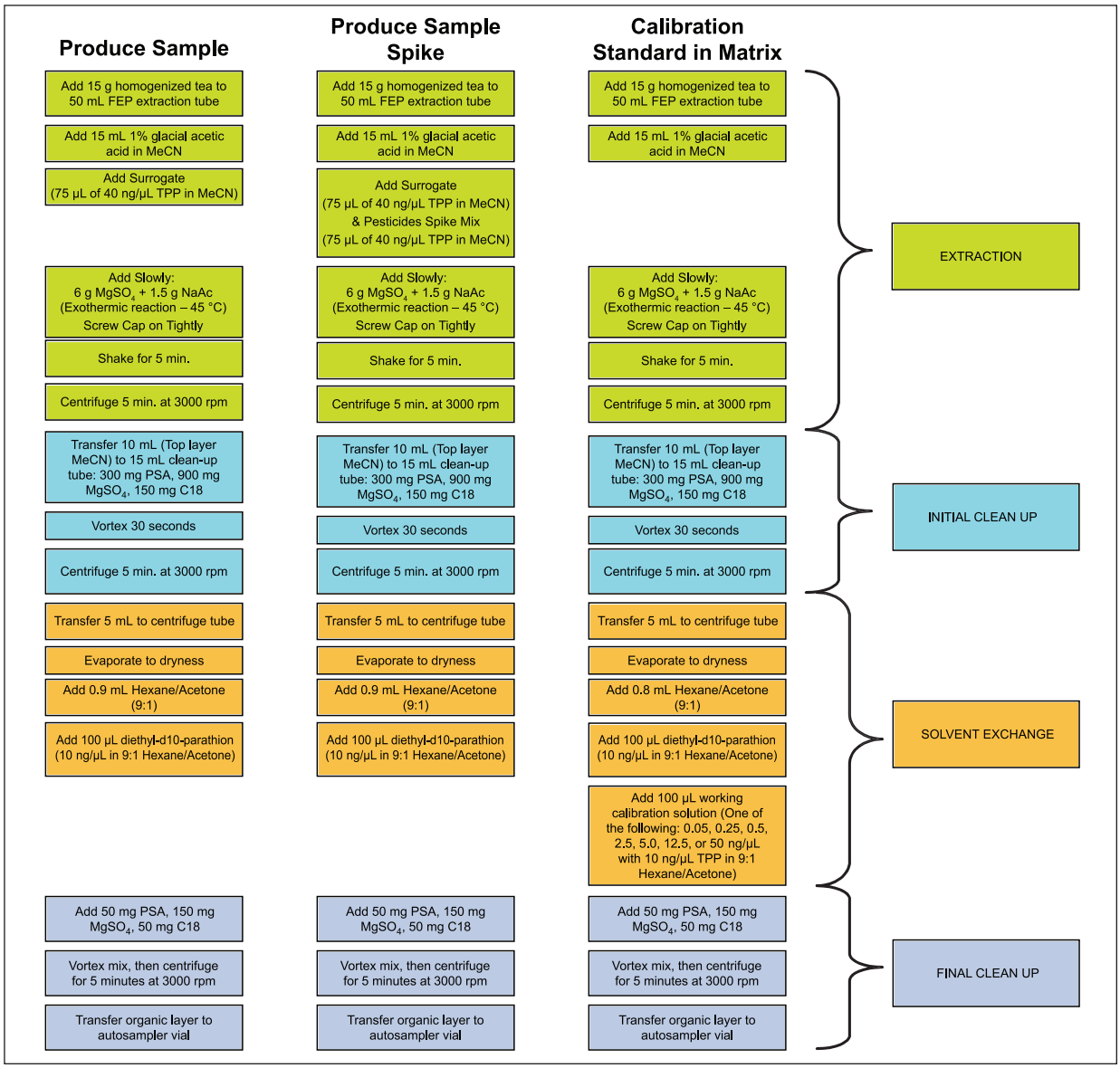

Figure 1: Flow diagram of QuEChERS sample preparation steps

The tube was capped and vortexed for 30 seconds. The cap was removed and the powder reagents were poured slowly into the MeCN layer. The cap was tightened securely on the 50 mL extraction tube, and was vortexed for 30 seconds until all of the powder reagents were mixed with the liquid layers. The tube was placed on a mechanical shaker for 5 minutes and then centrifuged for 5 minutes at 3000 rpm. Next, 11 mL of the top MeCN layer was removed and transferred to a 15 mL clean-up tube. This tube was capped and vortexed for 30 seconds and centrifuged for 5 minutes at 3000 rpm. A 5 mL aliquot of the top layer was transferred into a clean test tube for solvent exchange.

Solvent Exchange

The 5 mL aliquot of cleaned-up extract was evaporated to dryness with a gentle stream of nitrogen at 40 °C in about two hours. A film formed on top of the solvent layer and samples required mixing to break the film and continue the evaporation process. Care was taken to remove the tube immediately when dried. Approximately 1 mL of extracted compounds from the tea remained in the tubes after evaporation. A 900 µL aliquot of hexane/acetone (9:1) was added and 100 µL of the internal standard, d10-parathion, was spiked into the organic solution. The tube was capped and vortexed for 15 seconds. The 1 mL of extract was transferred to a 2 mL clean-up tube, capped tightly, and vortexed for 30 seconds. After centrifuging for 5 minutes at 3000 rpm, 200 µL of the lightly colored extract was transferred to an autosampler vial with a small glass insert for injection on the ITQ 700™. The individual calibration levels were spiked into each extract for the calibration curve in matrix before the final cleanup step (Figure 1).

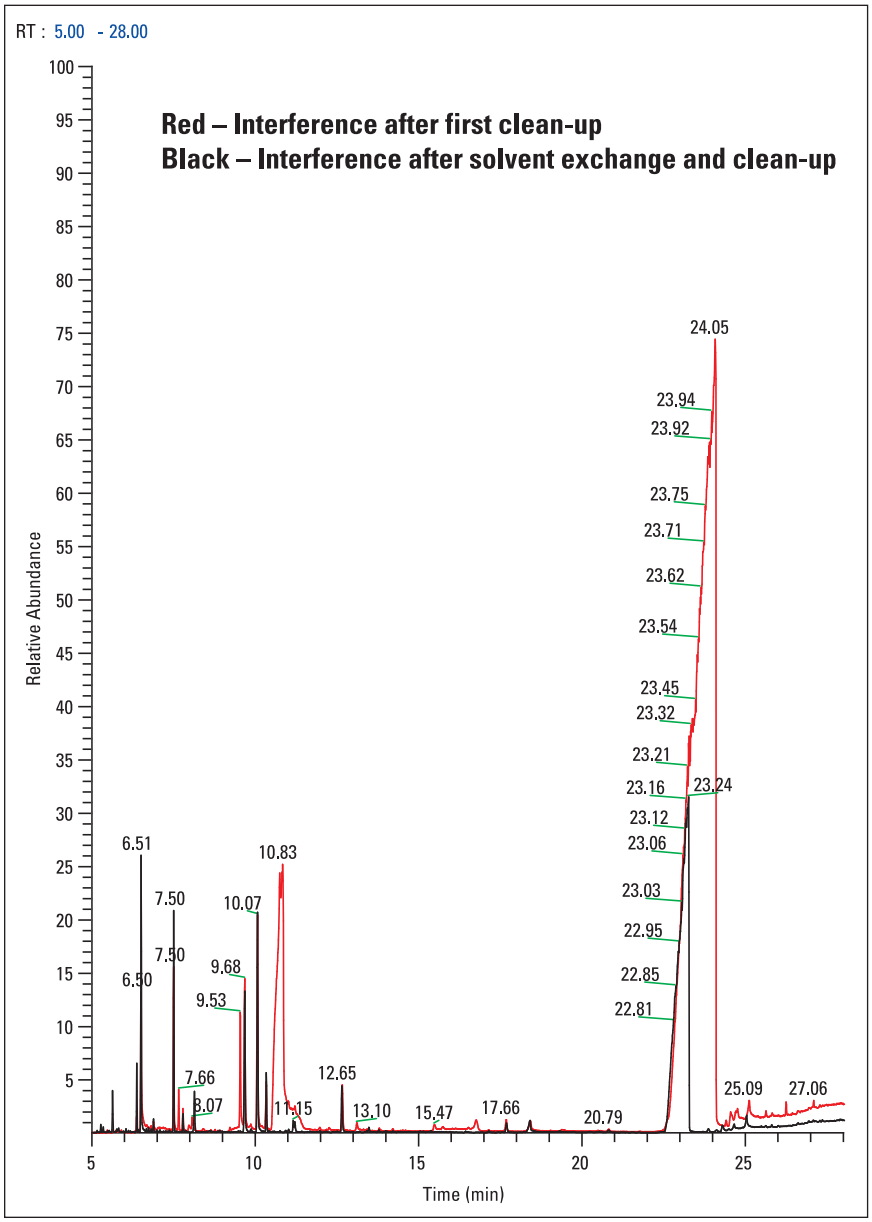

Figure 2: Comparison of a single cleanup step (red) against solvent exchange/final cleanup (black)

Injection

The ITQ 700 is paired with the Thermo Scientific FOCUS GC gas chromatograph, which is a single-channel GC with a standard split/splitless (SSL) injection port. The SSL inlet temperature was set to 250 °C. A 5 mm ID splitless liner with a volume of 1.6 mL was selected for the surged pressure injection. For the surge splitless injection, the inlet pressure was held at an elevated pressure of 250 kPa for the 0.5 minute injection (splitless) time. This technique reduces the vapor cloud of a 2 µL injection from 0.37 mL to 0.19 mL. At an elevated injection flow rate of 4.6 mL/ min, the liner was swept several times during injection. The target compounds moved through the inlet so rapidly that they had less time to interact with the inside walls of the liner. This minimized the amount of breakdown of the more fragile pesticides.

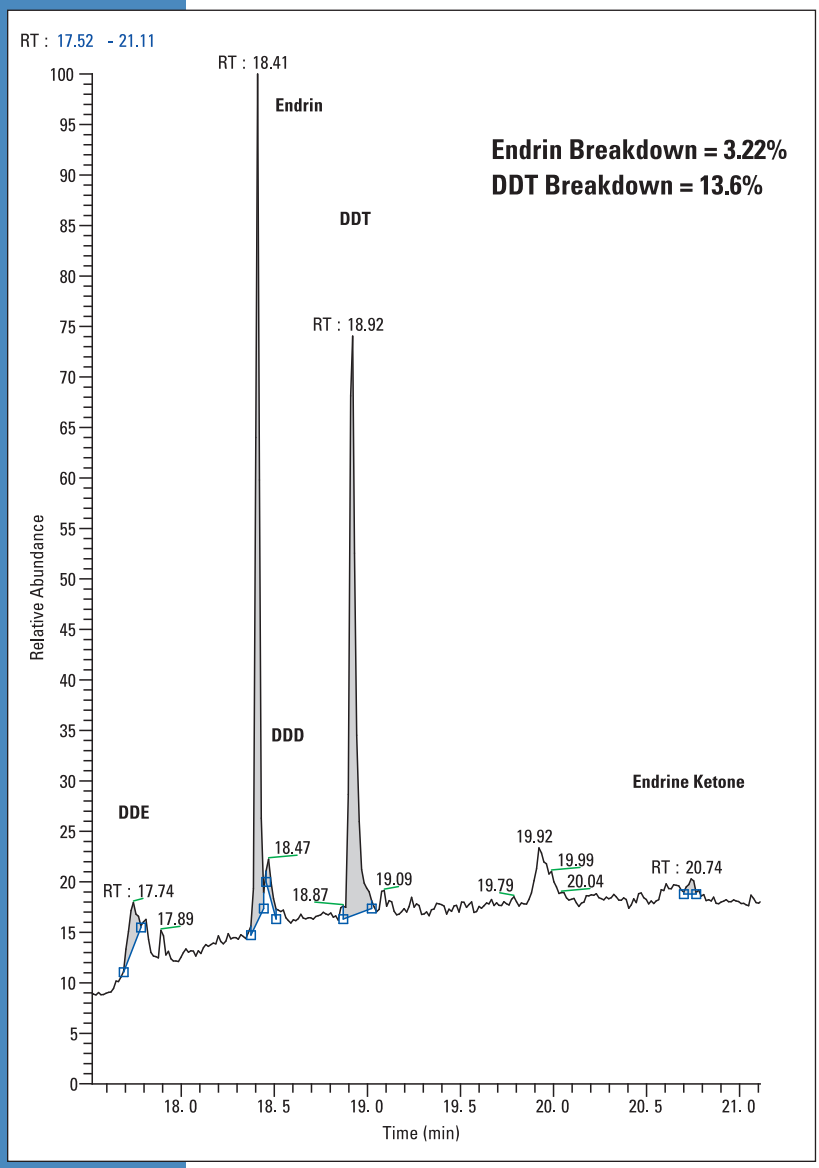

Figure 3: System performance check analysis demonstrating endrin breakdown < 5% and DDT breakdown < 15%

A Performance Solution consisting of endrin and 4,4'-DDT was analyzed as a daily check to determine system activity. The analysis of endrin, DDT, and their breakdown products as part of daily quality control can alert the analyst that the system has developed active sites and maintenance is needed. Without performing a breakdown analysis the laboratory may need to continually maintain the equipment and replace consumables, even when it may not be needed. Monitoring breakdown can decrease the cost of running the analysis and save significant amounts of time.

Endrin breakdown is determined by adding up the response for the two breakdown products: endrin aldehyde and endrin ketone and dividing by the total response for the breakdown products and endrin in percent. The breakdown products of DDT are DDE and DDD and are calculated similarly. The breakdown check results showed < 15% breakdown for both compounds on a daily basis. For routine use the liner would be changed when the breakdown of either compound reaches > 20%. The injection port liner tested showed very good results over a long period of time without the need for maintenance (Figure 3).

Separation

Chromatographic separation was achieved by using a 35% diphenyl/65% dimethyl polysiloxane column (0.25 mm x 30 meter, and a film thickness of 0.25 µm with a 5 m guard column). This column was chosen to improve the resolution of the more polar compounds. Some interactions within the stationary phase showed a loss of some pesticides at concentrations below 100 pg. The oven was programmed as follows: Initial Temp: 40 °C, 1.5 min, 25 °C/min to 150 °C, 0.0 min, 5 °C/min to 200 °C, 7.5 min, 25 °C/min to 290 °C with a final hold time of 12 min and a constant column flow rate of 1 mL/min. The entire set of instrument parameters is listed in Table 2.

Detection

The detection of the pesticides was performed using the ITQ 700 ion trap mass spectrometer with optional MS^n mode and a variable damping gas option. The MS/MS scan mode offers significantly enhanced selectivity over scanning modes such as full scan and selected ion monitoring (SIM). The ITQ 700 operated in the MS/MS mode generates unique product ion spectra by collision induced fragmentation of each of the detected pesticides. Because of the highly effective elimination of matrix interfering ions, more accurate results are produced at the lower levels. The MS^n parameters for the ITQ 700 are listed in Table 3. Figures 4 and 5 show a comparison between a Full Scan TIC and MS/MS extracted ion profile.

AS 3000 II Autosampler	
Sample Volume	2 µL
Plunger Strokes	5
Viscous Sample	No
Sampling Depth in Vial	Bottom
Injection Depth	Standard
Pre-inject Dwell Time	0
Post-inject Dwell Time	0
Pre-inject Solvent Wash Vial Position	A + B
Pre-inject Solvent Wash Cycles	3
Sample Rinses	3
Post-inject Solvent	A
Post-inject Solvent Cycles	3

FOCUS™ GC	
Column	TRACE TR-Pesticide III 35% diphenyl/65% dimethyl polysiloxane, 0.2 5 mm x 30 m x 0.25 µm w/ 5 m guard column
Column Constant Flow	1 mL/min
Oven Program	40 °C, 1.5 min, 25 °C/min; 150 °C, 0.0 min, 5 °C/min; 200 °C, 7.5 min, 25 °C/min; 290 °C, 12 min
S/SL Temperature	250 °C
S/SL Mode	Splitless with Surge Pressure
Surge Pressure	250 kPa
Inject Time	0.5 min
Split Flow	50 mL/min
Transferline Temperature	290 °C

ITQ Mass Spectrometer	
Damping Gas Flow	2
Source Temperature	250 °C
Ion Volume	EI
Emission Current	250 µA
Detector Gain	3 (1421 V)
Lens 1	-25V
Lens 3	-25V
Gate Lens On	-100
Gate Lens Off	100
Electron Lens On	15V
Electron Lens Off	85
Electron Energy	-70 eV
Trap Offset	-10
Waveforms	Off

Table 2: Selected instrument parameters for the ITQ 700 GC-ion trap MS

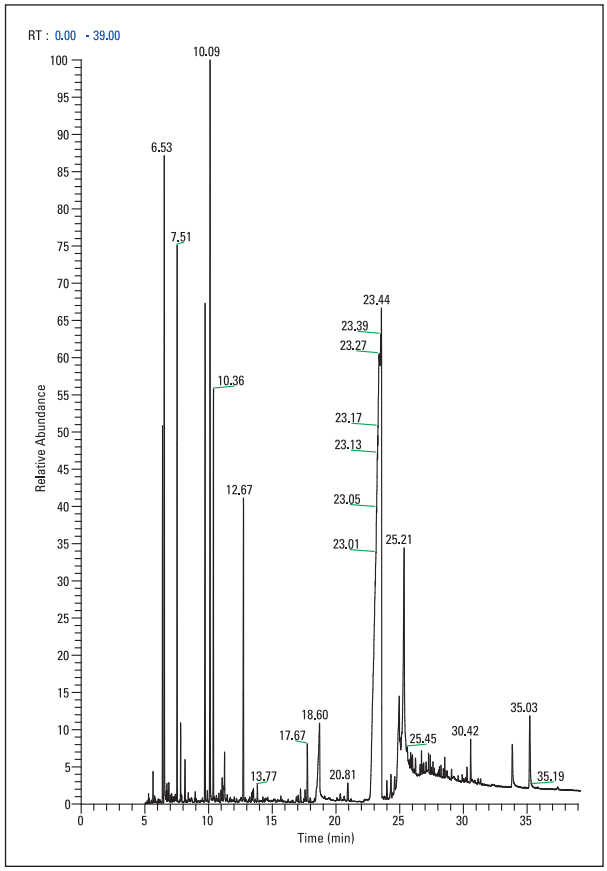

Figure 4: Full scan chromatogram of 600 ng/g pesticide spike in tea matrix

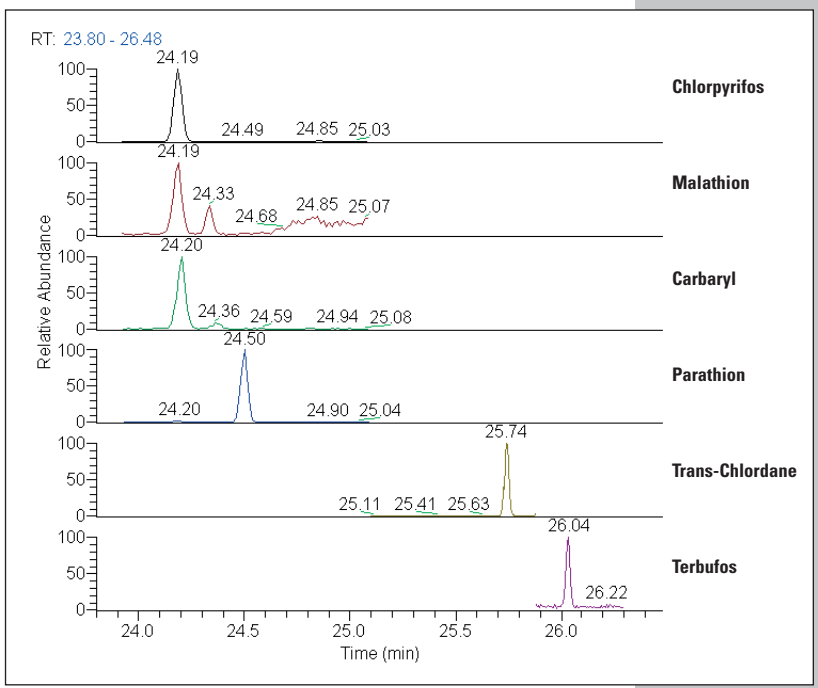

Figure 5: MS/MS scan of 600 ng/g pesticides in tea matrix, highlighting the elution range of 24 to 26 minutes

Compound	RT (min)	Precursor *(m/z)*	Width (amu)	Collision Energy (V)	Max. Excitation Energy (q)	Range *(m/z)*	Product Ion *(m/z)*	Qualifiers *(m/z)*
Dichlorvos	8.48	185	1	3	0.225	53-195	93	131, 109, 170, 63
Molinate	13.05	126	2	3	0.3	45-136	98	83, 55, 82, 81
Trifluralin	13.34	264	2	3	0.225	150-274	206	188, 160, 171, 177
Ethoprophos	14.57	158	2	2	0.225	84-168	114	130, 94, 140
Di-allate	15.45	234	3	3	0.225	140-244	192	150,193
Phorate	15.73	231	2	3	0.225	165-241	203	175, 185
Propyzamide (Pronamide)	16.86	173	2	3	0.225	135-183	145	146
Atrazine	17.42	200	6	4	0.225	84-210	122	132, 94, 134, 158
Diazanon	17.51	179	1	4	0.225	86-189	137	164, 138, 161, 96
Gamma BHC (Lindane)	17.92	219	4	3	0.225	171-229	181	183, 182, 184
Aldrin	22.15	263	1	5	0.225	217-273	229	228, 227, 230, 249
Metribuzin	23.69	198	2	4	0.225	93-208	151	103, 110, 153, 128
Dursban (Chlorpyrifos)	24.16	314	5	3	0.225	248-324	286	258, 287, 288, 285
Malathion	24.16	173	3	4	0.225	125-183	136	145, 137, 138, 135
Sevin (Carbaryl)	24.16	144	1	3	0.3	105-154	116	115
d-10 Parathion	24.34	301	2	3	0.225	105-311	269	147, 115, 148, 271
Parathion	24.49	291	4	3	0.225	99-301	142	263, 137, 109, 114
trans-Chlordane	25.73	375	4	4	0.225	256-385	301	266, 337, 303, 339
Terbufos	26.02	199	7	3	0.225	133-209	171	172, 153, 143, 173
cis-Chlordane	26.08	373	5	4	0.225	254-383	301	337, 299, 264, 335
Bifenthrin	28.29	181	7	4	0.225	143-191	166	165, 167, 178, 153
cis-Permethrin	30.99	183	3	4	0.225	143-193	168	165, 155, 153, 181
trans-Permethrin	31.19	183	3	4	0.225	143-193	168	165, 155, 153, 181

Table 3: MS/MS parameters for pesticides in tea

Results and Discussion

Linearity

The calibration curve was spiked into the tea matrix. Levels ranged from 1 ng/g to 1200 ng/g, depending on the compound and its MRL in green tea. The linearity for most compounds was $R^2 > 0.995$. The results of the linearity are shown in Table 4. Figures 6 and 7 are two examples of calibration curves.

Limits of Detection and Quantitation

The actual LOD and LOQ were determined by preparing matrix spikes at a level near or below the MRL. Concentrations of 7.5, 15, 37.5, 75, or 300 ng/g were analyzed in seven matrix samples and the LOD and LOQ calculated from these results by multiplying the standard deviation by 3.143 and 10 respectively. The results are shown in Table 5. These results exhibit that this method is able to meet or exceed the MRL requirements for most of the compounds, even at the most stringent level.

Method Validation Results

The method validation calculations were performed on five matrix samples spiked at a concentration of 37.5, 75, 150, 225, 600, or 1200 ng/g. Samples had an average of 104% recovery with an average % RSD of 10.8%. MVD results for selected concentrations are shown in Table 6.

Conclusions

The ITQ 700 GC-ion trap MS was thoroughly evaluated and showed excellent accuracy at low concentrations for a large number of pesticide residues analyzed in green tea. Using the instrument's MS^n functionality allows the user to identify, confirm, and quantify in one analytical run. The injector demonstrated low endrin and DDT breakdown (< 15%) on a daily basis, proving that the system can analyze active compounds without the need for continual, expensive, and time-consuming maintenance. Calibration curves for most pesticides studied met a linear least squares calibration with a correlation coefficient of $R^2 > 0.995$. The Method Validation Study generated an average % RSD of 10.8% for five replicate analyses at 37.5, 75, 150, 225, 600, or 1200 ng/g and a calculated average LOD of 14 ng/g in tea based on 7 replicate analyses of 7.5, 15, 37.5, 75, or 300 ng/g These results demonstrate that the ITQ 700 can comply with international regulations for the control of pesticides in tea.

Compound	(R^2)	Compound	(R^2)	Compound	(R^2)
Dichlorvos	0.9990	Gamma BHC (Lindane)	0.9949	*cis*-Chlordane	0.9944
Molinate	0.9996	Aldrin	0.9879	Bifenthrin	1.0000
Trifluralin	0.9994	Metribuzin	0.9966	*cis*-Permethrin	0.9993
Ethoprophos	0.9997	Dursban (Chlorpyrifos)	0.9998	*trans*-Permethrin	0.9998
Di-allate	0.9996	Malathion	0.9998	**Average**	**0.9989**
Phorate	0.9979	Sevin (Carbaryl)	0.9997		
Propyzamide (Pronamide)	0.9985	Parathion	0.9993		
Atrazine	0.9993	*trans*-Chlordane	0.9958		
Diazanon	0.9917	Terbufos	0.9993		

Table 4: Calibration curve results, demonstrating excellent linearity with average R^2 of 0.9978

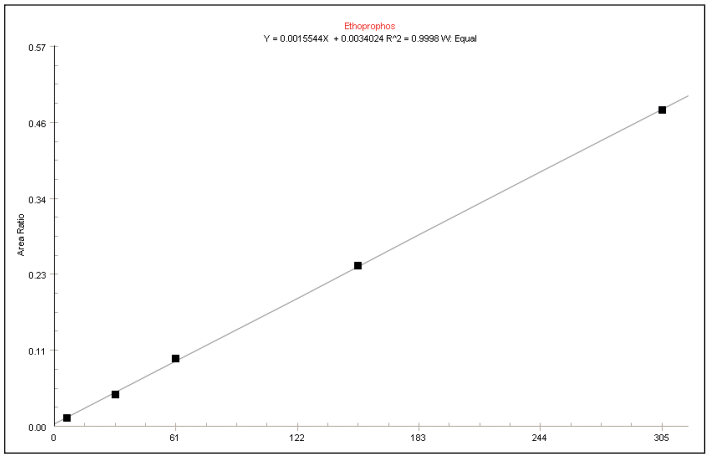

Figure 6: Calibration curve for Ethoprophos in tea

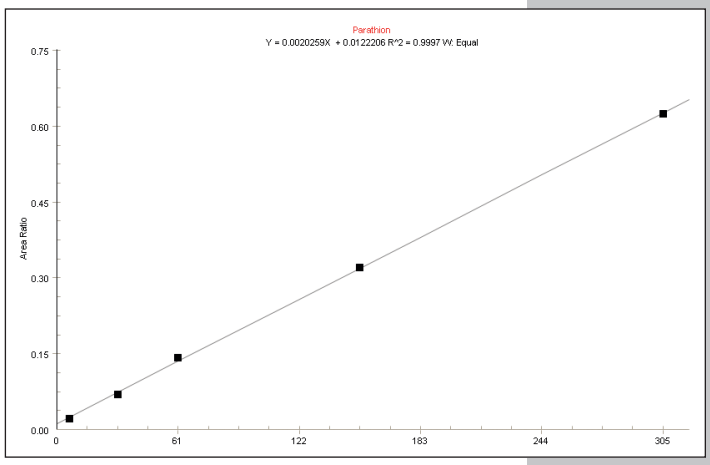

Figure 7: Calibration curve for Parathion in tea matrix

Component	Avg Conc (ng/g)	Std. Dev.	% RSD	LOD (ng/g)	LOQ (ng/g)	Japan[2] MRL (ng/g)	EU[3] MRL (ng/g)	EU[3] LOD (ng/g)	WHO[1] MRL (ng/g)
Dichlorvos	14	1.57	11.3	5	16	100	100	100	
Molinate	9	0.75	8.7	2	7	20			
Trifluralin	7	0.44	6.0	1	4	50			
Ethoprophos	6	0.89	14.0	3	9	5			
Di-allate	43	4.03	9.3	13	40	100	100	100	
Phorate	9	0.59	6.6	2	6	100	100	100	
Propyzamide (Pronamide)	11	1.04	9.6	3	10	50	50	50	
Atrazine	6	0.46	7.6	1	5	100	100	100	
Diazanon	13	0.60	4.7	2	6	100	50	50	
Gamma BHC (Lindane)	18	0.94	5.3	3	9	50	50	50	
Aldrin	7	1.70	25.2	5	17	ND	20	20	
Metribuzin	36	6.04	16.9	19	60	100			
Dursban (Chlorpyrifos)	19	2.80	14.7	9	28	10,000	100	100	2,000
Malathion	336	34.83	10.4	109	348	500	500		
Sevin (Carbaryl)	27	4.48	16.9	14	45	1000			
Parathion	46	7.82	17.2	25	78	300	100	100	
trans-Chlordane	8	1.21	14.8	4	12	20			
Terbufos	43	4.38	10.3	14	44	5			
cis-Chlordane	7	0.72	9.7	2	7	20			
Bifenthrin	32	6.09	19.0	19	61	25,000	5,000	100	
cis-Permethrin	78	7.71	9.9	24	77	20,000	100	100	20,000
trans-Permethrin	78	9.41	9.4	30	94	20,000	100	100	20,000
Average			**11.7**						

1. CODEX alimentarius (www.codexalimentarius.net/mrls/pesticides/jsp/pest-q-e.jsp)

2. Japanese Food Chemical Research Foundation (www.m5.ws001.squarestart.ne.jp/foundation/search.html)

3. Informal coordination of MRLs established in Directives 76/895/EEC, 86/362/EEC, 86/363/EEC, and 90/642/EEC (5058/VI/98)

Table 5: Comparison of LODs and LOQs to selected MRLs from international agencies and reporting bodies

Component	Avg Conc	Theo Conc	% RSD	% Recovery
Dichlorvos	158	150	4.5	105
Molinate	64	75	6.8	85
Trifluralin	66	75	4.2	89
Ethoprophos	64	75	3.4	86
Di-allate	59	75	4.1	79
Phorate	60	75	4.3	80
Propyzamide (Pronamide)	133	150	7.9	88
Atrazine	61	75	5.5	82
Diazanon	49	37.5	8.4	130
Gamma BHC (Lindane)	81	75	5.0	108
Aldrin	36	37.5	13.7	96
Metribuzin	67	75	11.7	89
Dursban (Chlorpyrifos)	175	225	6.3	78
Malathion	730	600	13.3	122
Sevin (Carbaryl)	72	75	9.9	96
Parathion	75	75	9.4	100
trans-Chlordane	43	37.5	16.0	115
Terbufos	76	75	9.8	101
cis-Chlordane	39	37.5	9.9	105
Bifenthrin	65	600	11.6	109
cis-Permethrin	1236	1200	15.1	103
trans-Permethrin	1239	1200	11.6	103
Average			**10.8**	**104**

Table 6: Results of method validation study, showing good precision and high recoveries

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3. Commission Decision of August 12, 2002 Implementing Council Directive 96/23/EC Concerning the Performance of Analytical Methods and the Interpretation of Results, *Official Journal of European Communities*. 17.8.2002.
4. MRLs for tea as listed at *http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp*.

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Application Notebook

Additional Contaminants

Packaging Contaminants

Thermo SCIENTIFIC

Unattended Automated Standard Addition and Headspace Analysis for the Quantitative Determination of VOCs in Food Packaging

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Application Note 1010

Key Words
TRACE 1310 GC, ISQ GC-MS, TriPlus RSH, Volatile Organic Compounds in Food Packaging

Goal
To demonstrate fully automated quantitative determination of volatile components in food packaging

Introduction
Headspace analysis by means of a dedicated autosampler is a standard technique for the determination of volatile organic compounds (VOCs) possibly present in food packaging materials. The packaging sample is typically cut into square pieces and placed in headspace vials for incubation at a determined temperature before the headspace sampling. The main challenge with this kind of analysis is the quantification of volatile compounds that are present because these samples are typically layered solids that generate adsorption and migration effects. External calibration is not reliable because it does not consider the matrix effect, which is significant for these samples.

In contrast, the standard addition calibration is a reasonable quantification procedure for these difficult matrixes because it uses real sample for the calibration procedure. Until now, preparation of samples for the standard addition calibration has been performed off-line, typically executed manually by the operator before headspace analysis of the samples. This is a time-consuming and error-prone procedure, while performing sample preparation by means of the same robotic sampler used for headspace analysis enables the quantification sequence to be run automatically in an unattended way. Detection and quantification are performed by means of a gas chromatograph (GC) coupled with a single quadrupole mass spectrometer (MS).

Materials and Methods
Use the Thermo Scientific TRACE 1310 GC, coupled with the ISQ Single Quadrupole GC-MS, for analysis of the samples. Configure the GC with an instant connect split/splitless (SSL) module, operated in split mode.

The headspace incubation temperature is 80 °C and the incubation time is 15 min. The headspace injected volume is 1 mL. Use the Thermo Scientific TraceGOLD TG-624 GC Column (1.4 µm film thickness; 0.25mm ID; 60m length).

The VOCs standards solution is:

- Residual Solvents in Packaging Material Mixture 1, analytical standard, 7.14% (v/v) (Sigma-Aldrich®)
- Residual Solvents in Packaging Material Mixture 2, analytical standard, 9.09% (v/v) (Sigma-Aldrich)

Mix to obtain a single comprehensive stock solution.

Automatically perform subsequent sample preparation and injection steps using the Thermo Scientific TriPlus RSH Autosampler. The tool exchange capability and vortex mixing allow the dilution step, the standard addition step, and the headspace analysis step to be combined together in the same sequence.

Procedures
Automatic Standard Dilution Procedure
The stock solution is first automatically diluted 1/1000 with water by the TriPlus™ RSH autosampler. In this cycle, the autosampler takes the necessary aliquot of water and places it in an empty vial, adding the amount of stock solution to bring to final volume (in this case, 1 ml) using a different volume syringe.

The diluted solution obtained contains all the components in a concentration range of 0.035–0.045 µg/µL that is suitable for the determination of low levels of residual solvent in packaging material.

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Use the first calibration to check the linearity of the system and the accuracy of the overall sample preparation procedure. The autosampler automates this calibration by adding various volumes (from 1 to 5 µL) of diluted solution to a set of empty vials before the headspace analysis of the vials.

For quantification by means of the standard addition method, cut samples of foil packaging from a commercially produced croissant product into square pieces of 48 cm^2 each. Prepare five 20 mL sample vials by placing a foil piece in a headspace vial which is then crimped. For each sample, these five vials are then further prepared and analyzed by the TriPlus RSH autosampler.

The autosampler adds selected amounts of standard into the headspace vials, plus a volume of a solvent calculated to mantain constancy in the total volume of liquid in the vial. This ensures that the same conditions are kept constant across all vials.

For example, in this study the following volumes have been used:

- Vial 1 (sample + 7 µL water)
- Vial 2 (sample + 1 µL standard + 6 µL water)
- Vial 3 (sample + 3 µL standard + 4 µL water)
- Vial 4 (sample + 5 µL standard + 2 µL water)
- Vial 5 (sample + 7 µL standard)

Separations

Perform the sampling of the prepared vials by means of headspace injection on the TRACE™ 1310 GC coupled with the ISQ™ Single Quadrupole GC-MS system (Figure 1).

Perform analysis of the sample in full-scan mode for the identification of components. Run the quantification step in selected ion monitoring (SIM) mode of masses: 31, 43, 45, 55, 56, 59, and 91 *m/z*. Both modes alternating full-scan/SIM are performed simultaneously per each sample injection.

Use the TraceGOLD TG-624 column for separation of the VOCs.

Table 1 shows the GC and headspace parameters.

Figure 1. TriPlus RSH Autosampler and TRACE 1310 GC with the ISQ Single Quadrupole GC-MS system.

Table 1. GC and headspace parameters

Oven Method	
Initial Temperature (°C)	35
Initial Time (min)	4
Heating Rate (°C/min)	4
Final Temperature (°C)	200
Hold Time (min)	0
SSL Method	
Temperature (°C)	200
Mode	split
Split Flow (mL/min)	80
Carrier Method	
Mode	Helium, Constant Flow
Value (mL/min)	1
TriPlus RSH Method	
Sample Volume (µL)	1000
Incubation Time (min)	15
Incubation Temperature (°C)	80
Syringe Temperature (°C)	120

Results and Discussion

The results show good correlation factors and limits of quantification <0.01 mg/m² for the majority of the components analyzed.

Linearity of Standard Addition

Figure 2 reports the list of components and the overlay of chromatograms obtained for each volume of standard (from 1 to 5 µL) added into empty vials.

The linearity of the system and the limits of quantification for all components are derived by converting the data of addition volumes into an absolute amount expressed in mg/m² (assuming that the sample surface in the vial is 48 cm²). The graphs in Figure 3 show good linearity of the system. Quantification limits below 0.01 mg/m² have been calculated for all compounds except THF, ethanol, and ethoxy ethanol.

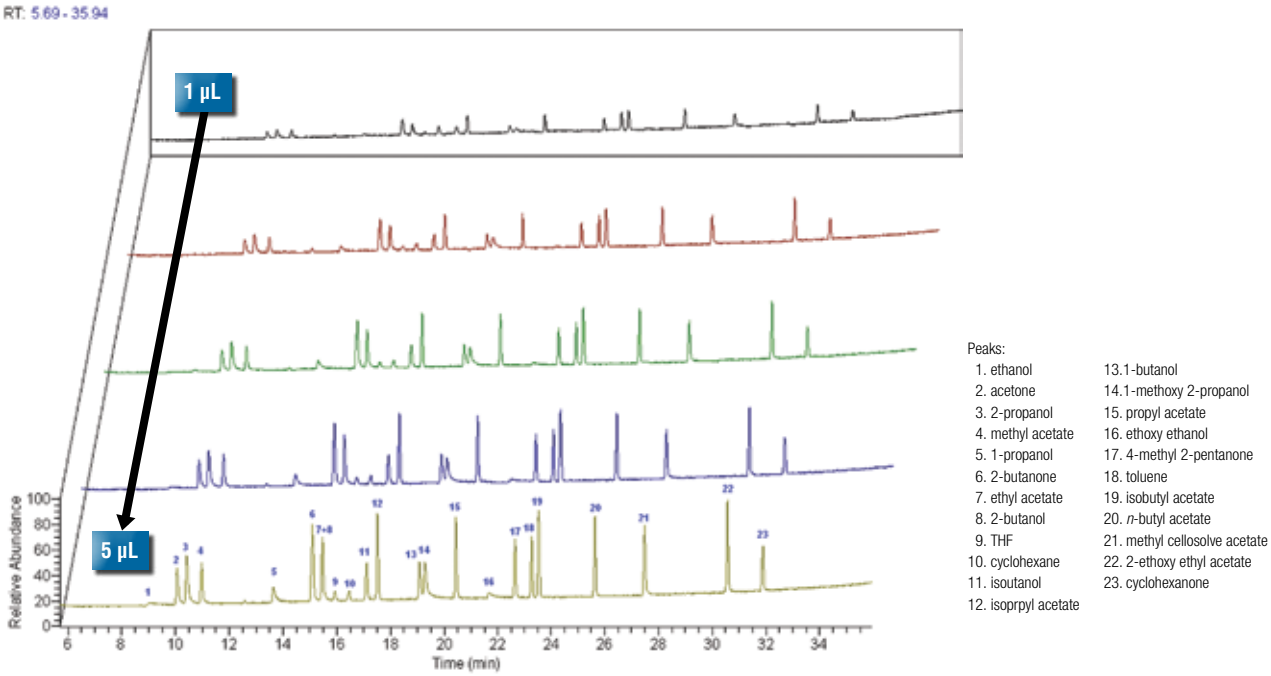

Figure 2. Component list and overlay of chromatograms in the elution order.

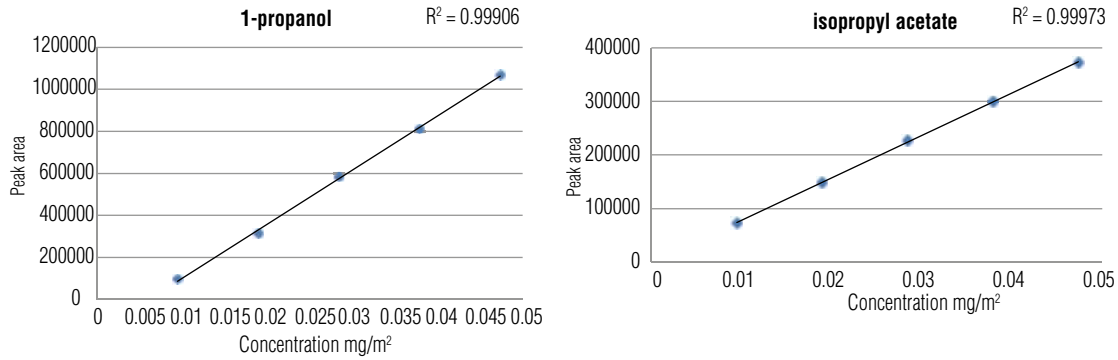

Figure 3. System linearity for 1-propanol and isopropyl acetate.

Sample Analysis (Components Identification)

A full-scan chromatogram of the packaging sample was performed in order to identify the components possibly present.

Figure 4 shows the presence of numerous residual solvents. The larger ethanol peak comes from the croissant itself where ethanol is used as a preservative.

Sample Analysis (Components Quantification)

Quantification performed by means of the standard addition method was carried out for the peaks identified in the chromatogram in Figure 4. Figure 5 shows the standard addition calibration curve for *n*-butyl acetate together with the results of quantification in the sample.

Table 2 presents the results of the quantification.

In Figure 6, the graphical representation of the quantification analysis shows that the two main residual solvents identified in the sample are 1-methoxy 2-propanol and 2-propanol. Their amounts do not exceed the level of 0.1 mg/m² each, while the other detected residual solvents have been quantified in the level of 0.01 mg/m² or lower.

Conclusion

A completely automated method for analyzing and quantifying VOCs in food packaging materials is presented. The combination of sample preparation steps and an analytical step in the same sequence allows high accuracy in quantification, high sample throughput, and minimizes error-prone manual manipulations. For the majority of residual solvents analyzed in this method, their individual limit of quantitation was below 0.01 mg/m².

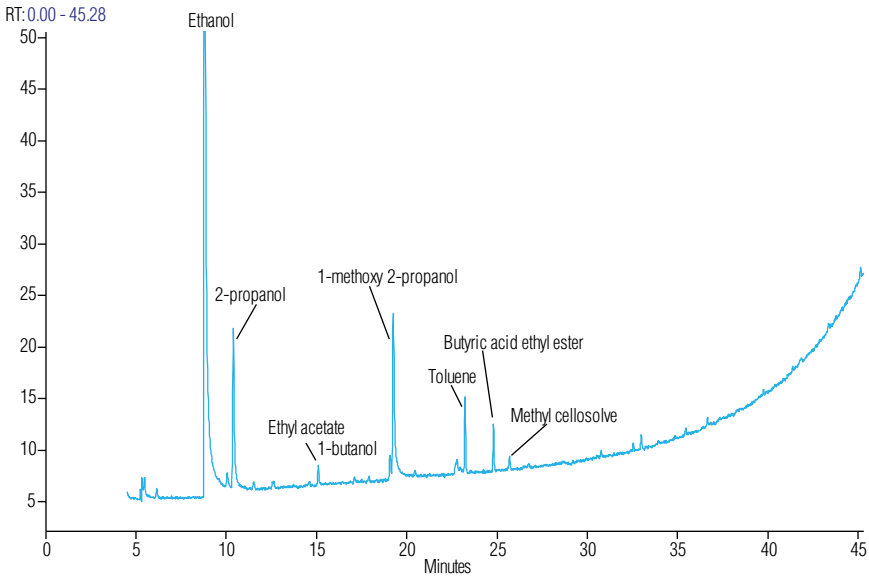

Figure 4. Full-scan sample analysis by headspace.

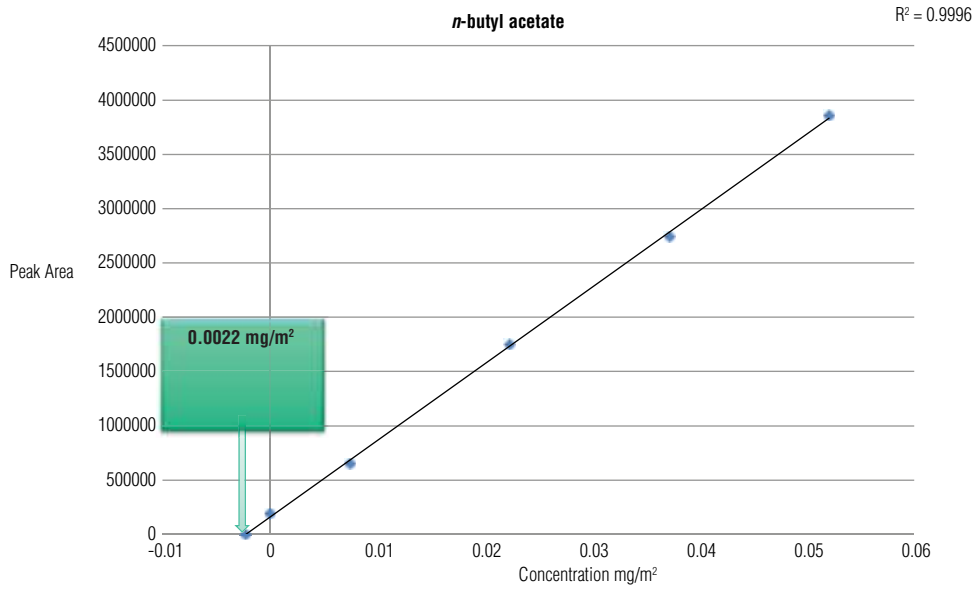

Figure 5. Quantitation via standard addition calibration for *n*-butyl acetate.

Table 2. Quantification results from a real croissant packaging foil sample of 48 cm^2 area

	µL added					
Component	**0**	**1**	**3**	**5**	**7**	
2-propanol	3337694	4162371	6363394	7060114	8101360	area
	0	0.009469	0.028406	0.047344	0.066281	concentration mg/m^2
	5.02E-02					calculated amount mg/m^2
ethyl acetate	302671	973347	2755297	4304253	6115361	area
	0	0.007438	0.022313	0.037188	0.052063	concentration mg/m^2
	2.00E-03					calculated amount mg/m^2
1-butanol	385906	761497	1708139	2203362	3191062	area
	0	0.007438	0.022313	0.037188	0.052063	concentration mg/m^2
	7.55E-03					calculated amount mg/m^2
1-methoxy 2-propanol	3873000	4854145	5683277	6258900	7857878	area
	0	0.009469	0.028406	0.047344	0.066281	concentration mg/m^2
	7.44E-02					calculated amount mg/m^2
4-methyl 2-pentanone	374131	837187	1642593	2555176	3209676	area
	0	0.009469	0.028406	0.047344	0.066281	concentration mg/m^2
	9.57E-03					calculated amount mg/m^2
toluene	1077304	1512873	2382486	2972530	3683690	area
	0	0.007438	0.022313	0.037188	0.052063	concentration mg/m^2
	2.31E-02					calculated amount mg/m^2
n-butyl acetate	190875	649615	1751135	2741910	3859582	area
	0	0.007438	0.022313	0.037188	0.052063	concentration mg/m^2
	2.26E-03					calculated amount mg/m^2

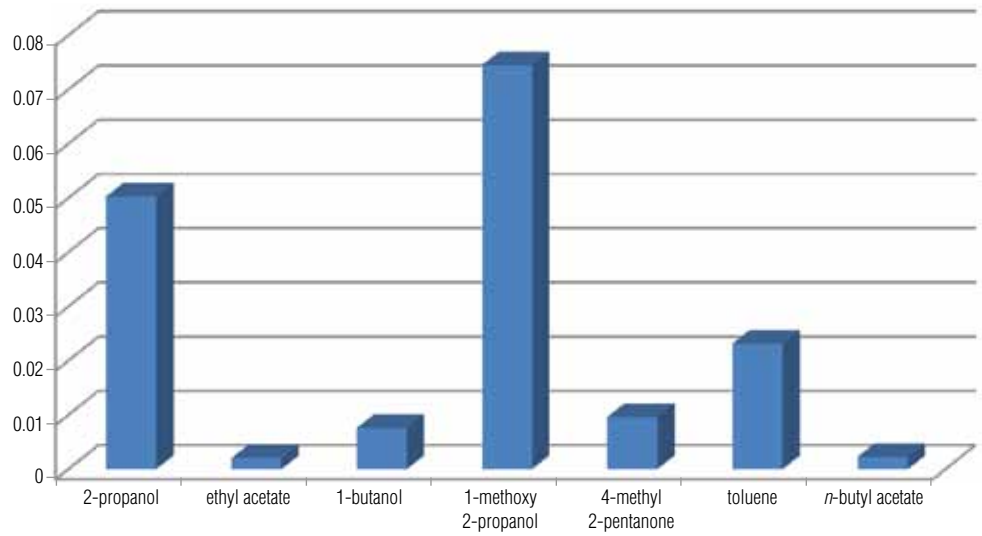

Figure 6. VOCs detected in croissant packaging and their relative distribution.

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Determination of Phthalates in Liquor Beverages by Single Quadrupole GC-MS

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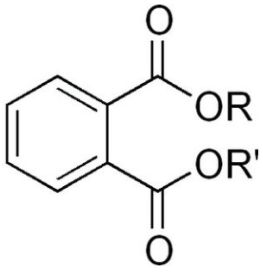
Introduction

Phthalates (Phthalate Acid Esters, PAEs) have widespread use in the polymer industry as plasticizers and softeners to increase the plasticity of polymer materials and their toughness and strength. They are chemically inert, have high density, low to medium volatility, high solubility in organic solvents, and are easily released to the environment during aging of polymer materials. Phthalates had been reported as functional solvents in the aromatic, essential oil, and even beverage industries. Phthalate plasticizers also migrate from plastic containers or closures into soft drinks and alcoholic beverages.

PAEs in the environment and food chain can act as hormones, simulate the body's natural endocrine responses, interfere with the normal role of hormones, and affect the body's most basic physiological control mechanisms. Phthalates are reported to cause carcinogenic, teratogenic, and mutagenic effects and constitute a health hazard to humans.



Phthalate residues in food and beverages are regulated internationally. The China Ministry of Health issued a public notice on June 1st, 2011, that phthalate esters are clearly prohibited as non-food substances for use in food. PAEs are introduced into the food chain primarily through food packaging material. Alcoholic beverages in plastic containers are a particular risk, since the containing ethanol provides a very good solubility for PAEs and is leaching the PAEs into the beverages from the plastic contact materials. The contamination risk increases with liquors having high ethanol content. On November 19th, 2012, Chinese media reported that, according to third-party testing, PAE plasticizer content in a well-known domestic liquor brand was up to 260% higher than the regulated level.

This study follows the China regulation GB/T 21911-2008 for the determining of phthalates in food[1].The sample preparation procedure was optimized from GB/T 21911-2008 with the ethanol removal from liquor beverages followed by an n-hexane extraction and gas chromatography/mass spectrometry (GC-MS) detection. The method is sensitive, rapid, and accurate, and covers a wide linear range to meet the need for trace level detection of phthalate esters in different types of beverages.

Experimental Conditions

Sample preparation

The sample used for this application was a white spirit, bought from a local liquor store. An accurate amount of 5.0 mL sample was transferred in a glass

centrifuge tube and then heated in a boiling water bath to remove the ethanol[2]. The heating time depends on the alcoholic strength of the spirit sample. Usually the tube was removed from the water bath with a residual volume of 2-3 mL. After cooling to room temperature, 2.0 mL of n-hexane was added, and the glass tube was shaken for extraction and left standing 5 minutes for phase separation. The supernatant was transferred to autosampler vials for analysis. Special attention is required that all glassware need to be cleaned with hexane and baked in a muffle furnace at about 450 °C in order to avoid contamination.

A commercial phthalate standard was used for method development. For optimization of the extraction procedure and recovery determination, one liquor sample was spiked with 4 mg/L concentration of the phthalate standard.

GC-MS instrument conditions

All measurements have been carried out using the Thermo Scientific™ ISQ Series single quadrupole GC-MS system with a Thermo Scientific™ TRACE™ 1310 GC equipped with the instant connect SSL injector module (split/splitless injector) and a Thermo Scientific AS 1310 liquid autosampler. The instrument conditions are listed in Tables 1 and 2.

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Table 1. GC conditions

Column type	Thermo Scientific™ TraceGOLD TG-35MS
Column dimensions (length × i.d. × film thickness)	30 m × 0.25 mm × 0.25 µm
	P/N 26094-1420
Injector type, Temperature	SSL, 280 °C
Injection mode, Volume	Splitless, 1 µL
Carrier gas, flow	Helium, constant flow 1 mL/min
Oven program	80 °C, 1 min
	10 °C/min to 280 °C
	280 °C, 10 min
Transfer line temperature	280 °C

Table 2. MS Conditions

Ionization	EI, 70 eV
Scan mode, range	Full Scan, 50-350 Da
Acquisition rate	0.2 s
Ion source temperature	280 °C

Sample Measurements

First, the elution order of the phthalate compounds was determined by analyzing a standard mixture at medium concentration. The spectra observed were compared with the NIST data base for identification and retention time determination.

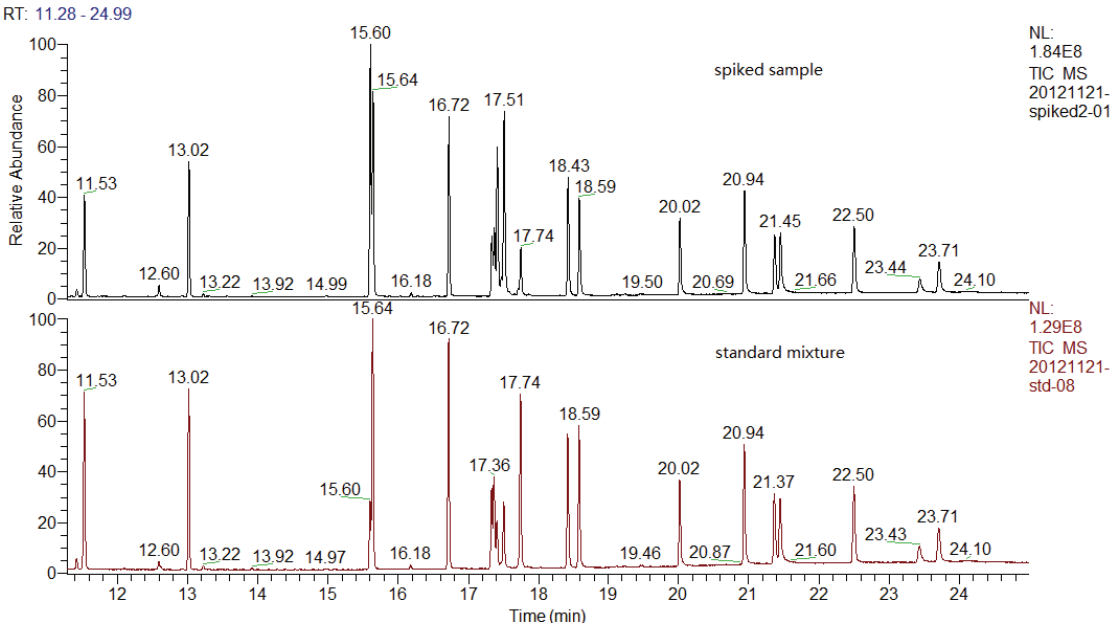


Figure1. Chromatograms of a spiked sample (top) and of the standard compounds run (bottom).

The compound quantitation was performed by selecting the most intense and unique ions of the compounds providing selective mass chromatograms for individual peak integration.

Finally, eight commercial liquor samples from a local liquor shop were prepared by the described sample preparation method for determining possible contamination by phthalate esters.

Optimization of the liquor sample extraction

Chinese liquor typically contains 30 to 60 vol% ethanol. Phthalate esters are highly soluble in ethanol, so the extraction of phthalate esters using n-hexane as solvent is less effective[2]. The removal of the major part of ethanol from the liquor before n-hexane extractionis necessary to avoid low recoveries.

Accurately measured 5.0 mL liquor samples were transferred into glass tubes. Then the standard solution was added to obtain a spiked solution at 0.80 mg/L concentration level. Figure 1 shows chromatograms of spiked sample and standard mixture runs. The experiment results were compared with and without ethanol removal. The results from the recovery experiment are shown in Table 3. After removal of ethanol before the extraction with n-hexane, good and consistent recoveries of the phthalate compounds in the range of 89-112% were obtained.

Table 3. Comparison of recovery of phthalates from liquor without and with prior removal of ethanol before extraction.

Compound	CAS #	Abbreviation	With outethanol removal Recovery (%)	With ethanol removal Recovery (%)
Dimethyl phthalate	131-11-3	DMP	60.0	102.0
Diethyl phthalate	84-66-2	DEP	35.4	107.0
Diisobutylphthalate	84-69-5	DIBP	99.5	94.4
Di-n-butyl phthalate	84-74-2	DBP	106.0	104.0
Di-(4-methyl-2-pentyl) phthalate	146-50-9	DMPP	99.7	95.1
Di-(2-methoxy)-ethyl phthalate	117-82-8	DMEP	3.38	88.8
Diamylphthalate	131-18-0	DPP	109.0	108.0
Di-(2-ethoxy)-ethyl phthalate	605-54-9	DEEP	13.6	103.0
Dihexylphthalate	68515-50-4	DHP	104.0	101.0
Butylbenzyl phthalate	85-68-7	BBP	88.4	108.0
Di-(2-ethylhexyl) phthalate	117-81-7	DEHP	106.0	108.0
Di-(2-butoxy)-ethylphthalate	117-83-9	DBEP	83.1	104.0
Dicyclohexyl phthalate	84-61-7	DCHP	94.8	102.0
Di-n-octylphthalate	117-84-0	DNOP	103.0	106.0
Diphenyl phthalate	84-62-8	DPhP	77.1	112.0
Dinonylphthalate	84-76-4	DNP	110.0	109.0

Results

In the following, the detection of five components of the phthalate standard mixture is shown as an example of the investigated PAE compounds listed in Table 3. Although the full scan chromatograms shown in Figures 2-7 give high background signals and include the elution of many other compounds dissolved in the spirit, the selective mass traces of the major phthalate ions allow a very good selectivity for reliable peak area integration.

The mass spectra shown in Figure 8 are taken for comparison to confirm the compound identity from the analysis of the spiked sample and the standard run.

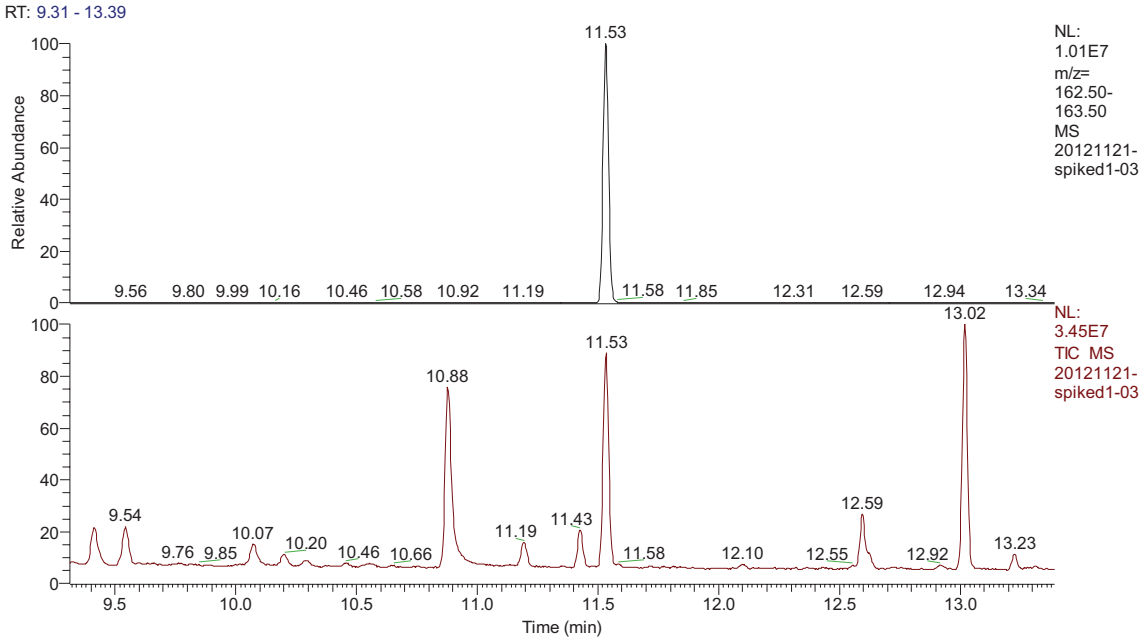


Figure 2. Dimethyl-phthalate chromatograms from spiked sample with the selective mass chromatogram (top) and the Full Scan trace (bottom) allowing the interference free peak area integration of the PAE compound.

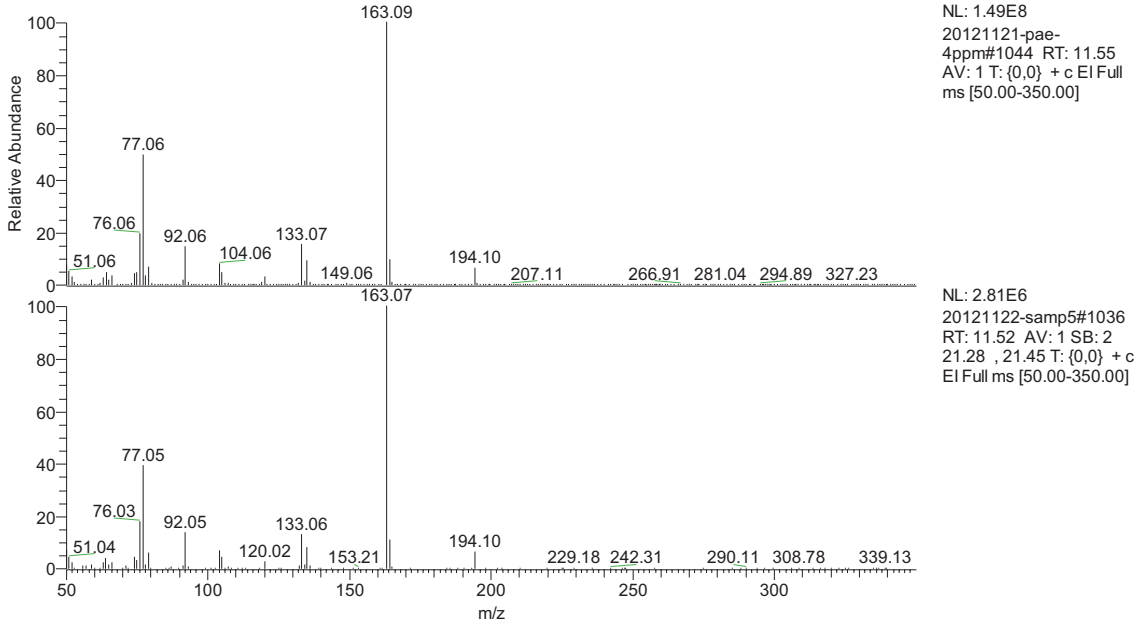


Figure 3. Dimethyl-phthalate spectra from standard (top) and sample (bottom).

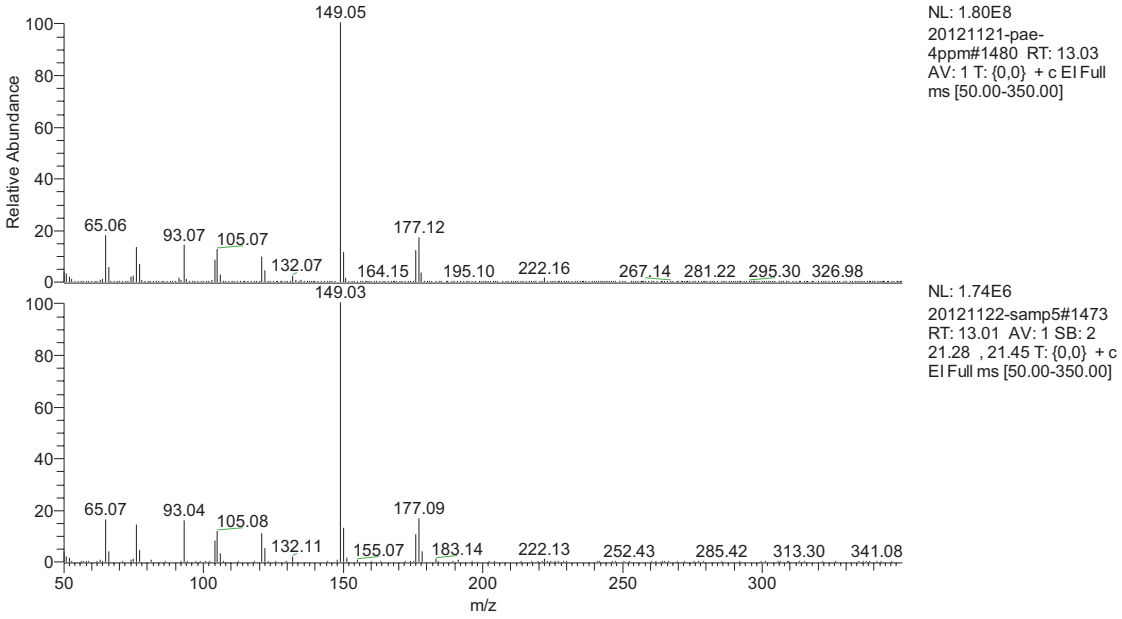


Figure 4. Diethyl-phthalate spectra from standard (top) and sample (bottom).

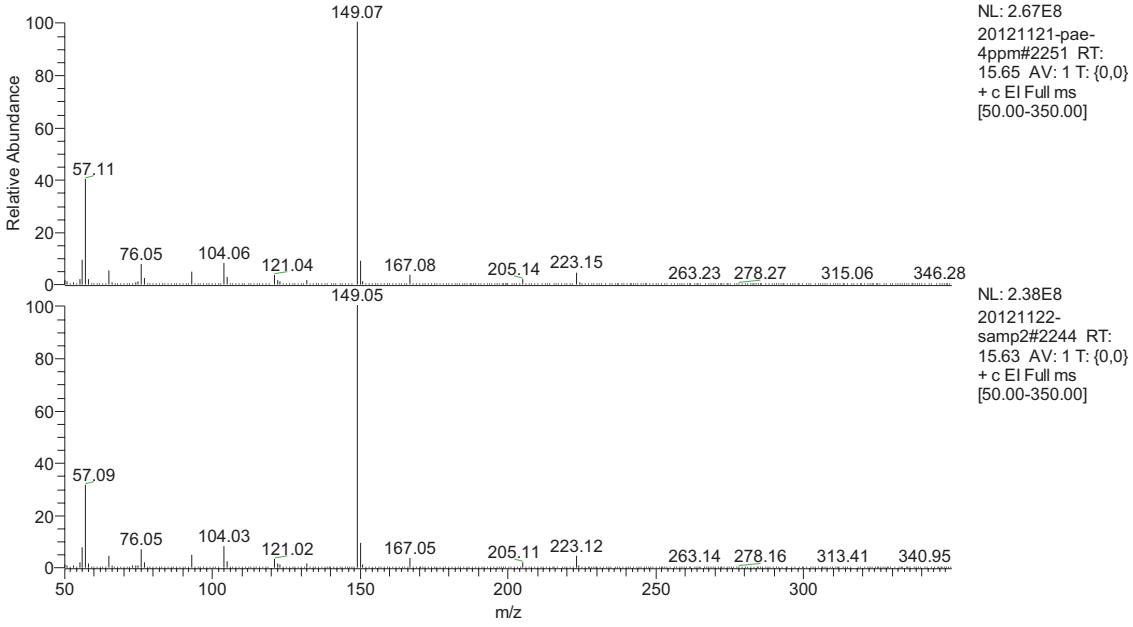


Figure 5. Di-isobutyl-phthalate spectra from standard (top) and sample (bottom).

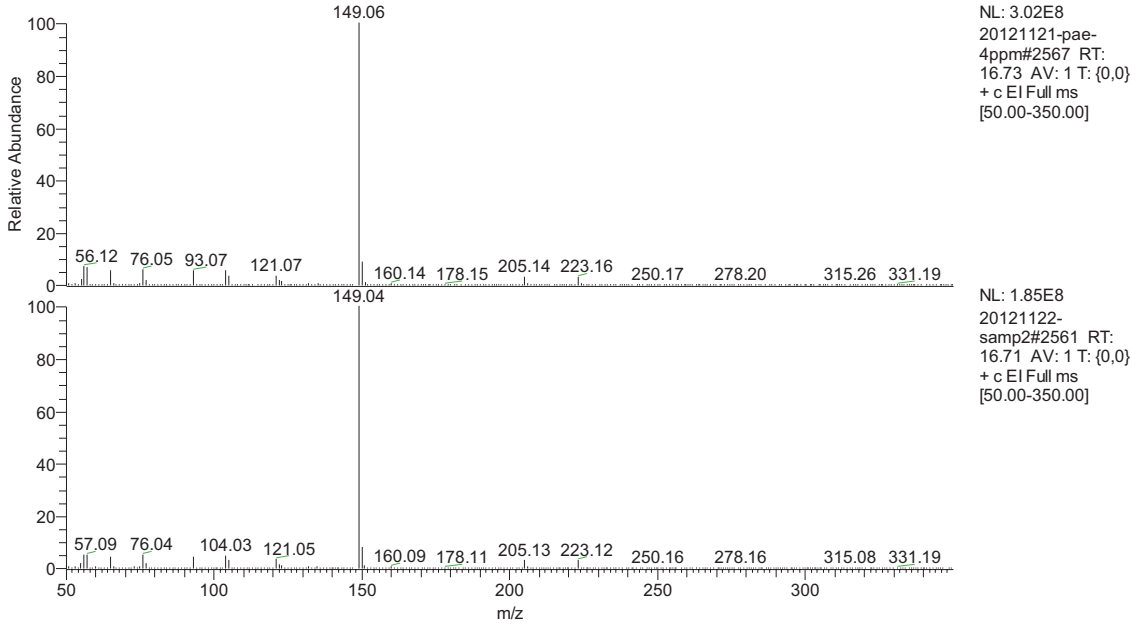


Figure 6.Dibutyl-phthalate spectra from standard (top) and sample (bottom).

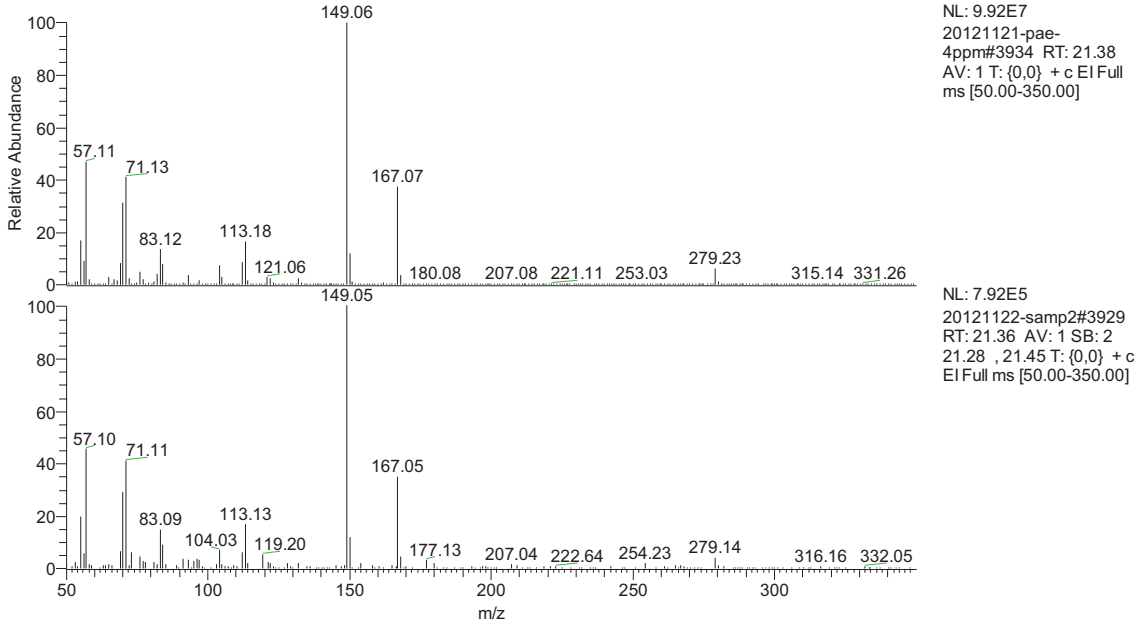


Figure 7. Di-(2-ethylhexyl) phthalate spectra from standard (top) and sample (bottom).

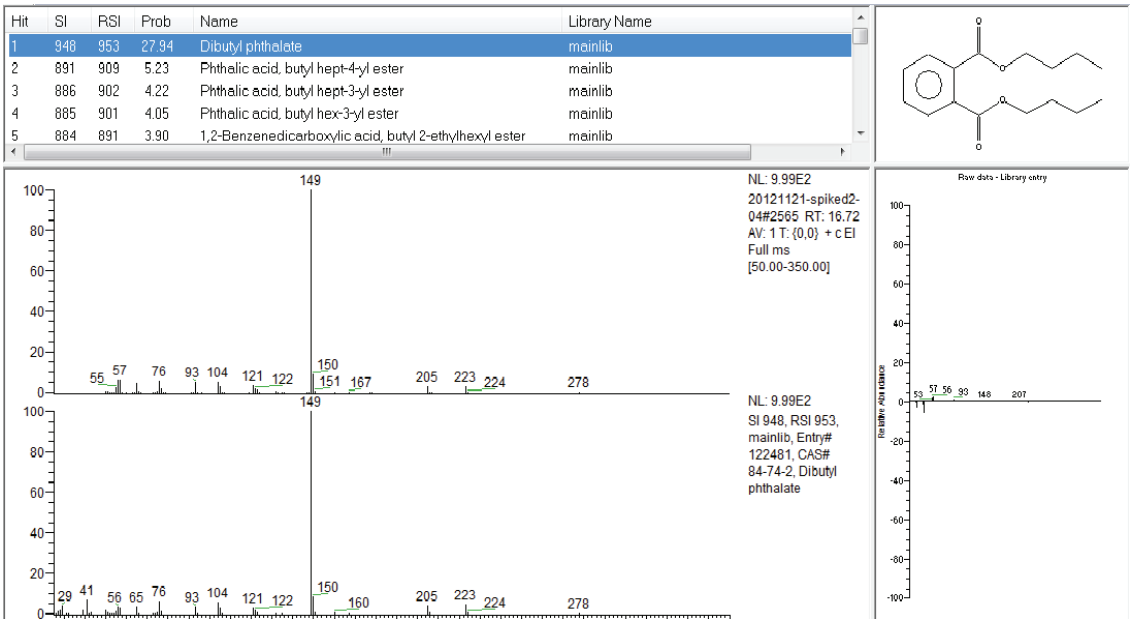


Figure 8. Comparison of spectra between the spiked sample (top) and NIST library (bottom) indicating a high match factors (Similarity Index SI, Reversed Search Index RSI).

Quantitation

A series of matrix spiked samples with five different concentrations was prepared in the range of 0.10 to 4.00 mg/L of the standard solution. The samples were injected in sequence from low to high concentration. The peak areas were calculated for the standard curve with linear regression of very good precision with an average R^2 value of 0.999 for all PAE compounds. The results for 15 phthalate esters show a very good linear relationship in the full calibration range of 0.10 to 4.00 mg/L.

The dinonyl-phthalates (DNP) create a special analytical challenge. The DNPs typically consist of a mixture of technical C9-isomers. Hence the response of DNP is distributed to individual isomers. The integration of the unresolved DNP peaks needs to be performed over a wider but constant retention-time range, as shown in Figure 9 from the applied Thermo Scientific™ TraceFinder™ software data processing. We could achieve a linear calibration range for DNP of 0.40 to 4.00 mg/L.

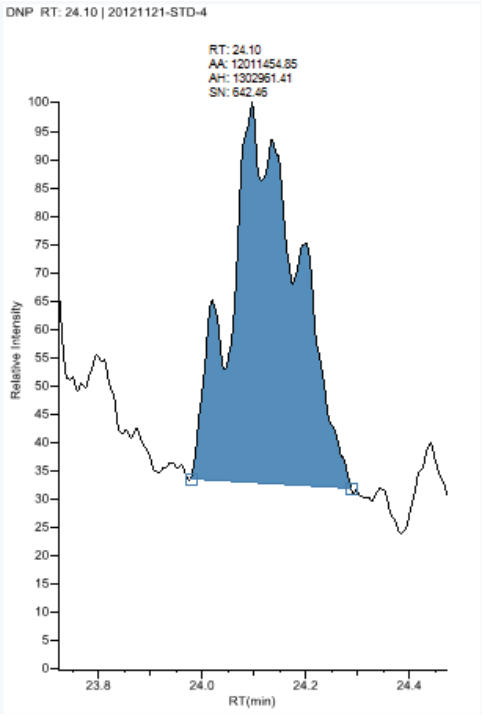


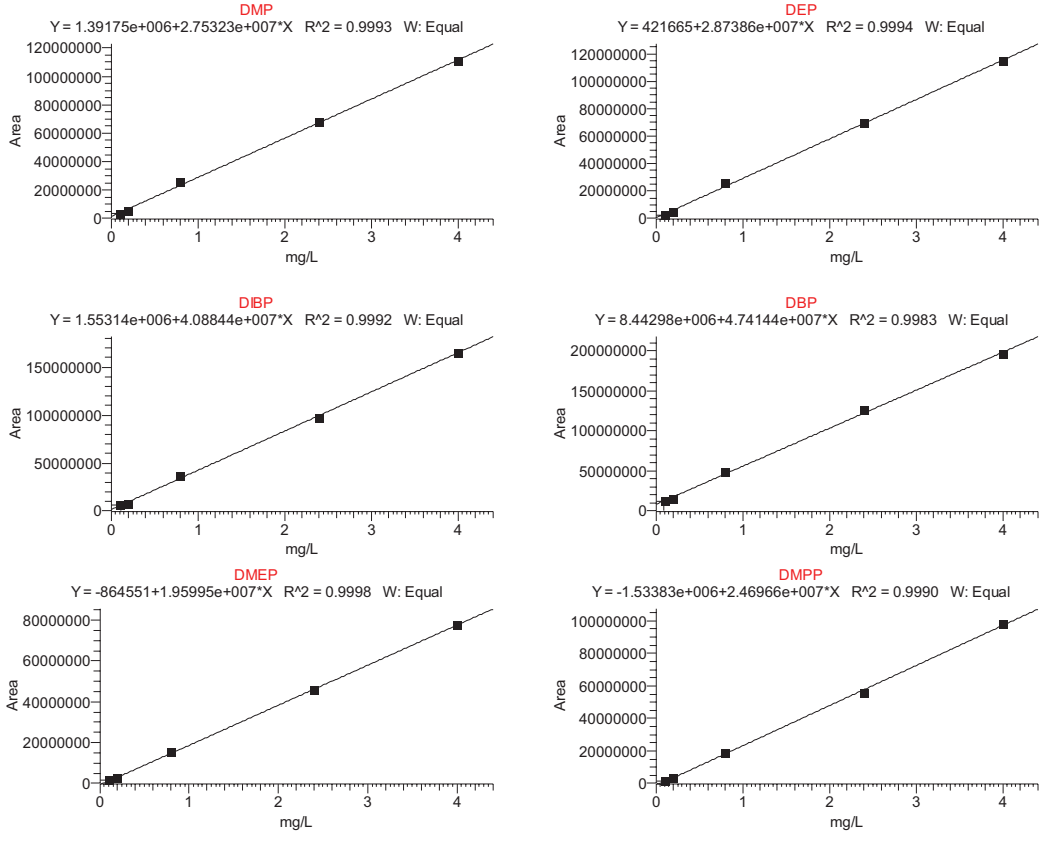
Figure 9. Quantitation peaks of the unresolved DNP isomers over a set retention time range using the TraceFinder quantitation software.

Sensitivity

The determination of the limit of detection (LOD) and limit of quantitation (LOQ) were based on the characteristic extracted ion mass chromatograms with a peak signal-to-noise ratio S/N ≥ 3 for LOD, and S/N ≥10 for LOQ, as given in Table 4. For the individual phthalate compounds. Figure 10 shows the calibration curves of 16 PAE compounds.

Table 3. Phthalate Quantitation - Linearrange withlimit of detection (LOD) andlimit of quantification (LOQ), average R^2 0.9990.

Compound name	Retention time [min]	Quantitation ion [m/z]	Linear range [mg/L]	Correlation coefficient R^2	LOD [µg/L]	LOQ [µg/L]
DMP	11.53	163	0.1-4.0	0.9994	0.1	0.3
DEP	13.02	149	0.1-4.0	0.9999	0.1	0.3
DIBP	15.64	149	0.1-4.0	0.9981	0.1	0.3
DBP	16.72	149	0.1-4.0	0.9986	0.1	0.3
DMPP	17.33/17.36	149	0.1-4.0	0.9993	0.2	0.6
DMEP	17.74	59	0.1-4.0	0.9984	0.2	0.6
DPP	18.43	149	0.1-4.0	0.9996	0.1	0.3
DEEP	18.59	72	0.1-4.0	0.9996	0.1	0.3
DHP	20.02	149	0.1-4.0	0.9990	0.1	0.3
BBP	20.94	149	0.1-4.0	0.9998	0.2	0.6
DEHP	21.37	149	0.1-4.0	0.9969	0.2	0.6
DBEP	21.45	149	0.1-4.0	0.9993	0.5	1.5
DCHP	22.50	149	0.1-4.0	0.9985	0.2	0.6
DOP	23.43	149	0.1-4.0	0.9998	0.5	1.5
DPhP	23.70	225	0.1-4.0	0.9988	0.2	0.6
DNP	24.0-24.4	149	0.4-4.0	0.9983	50	150



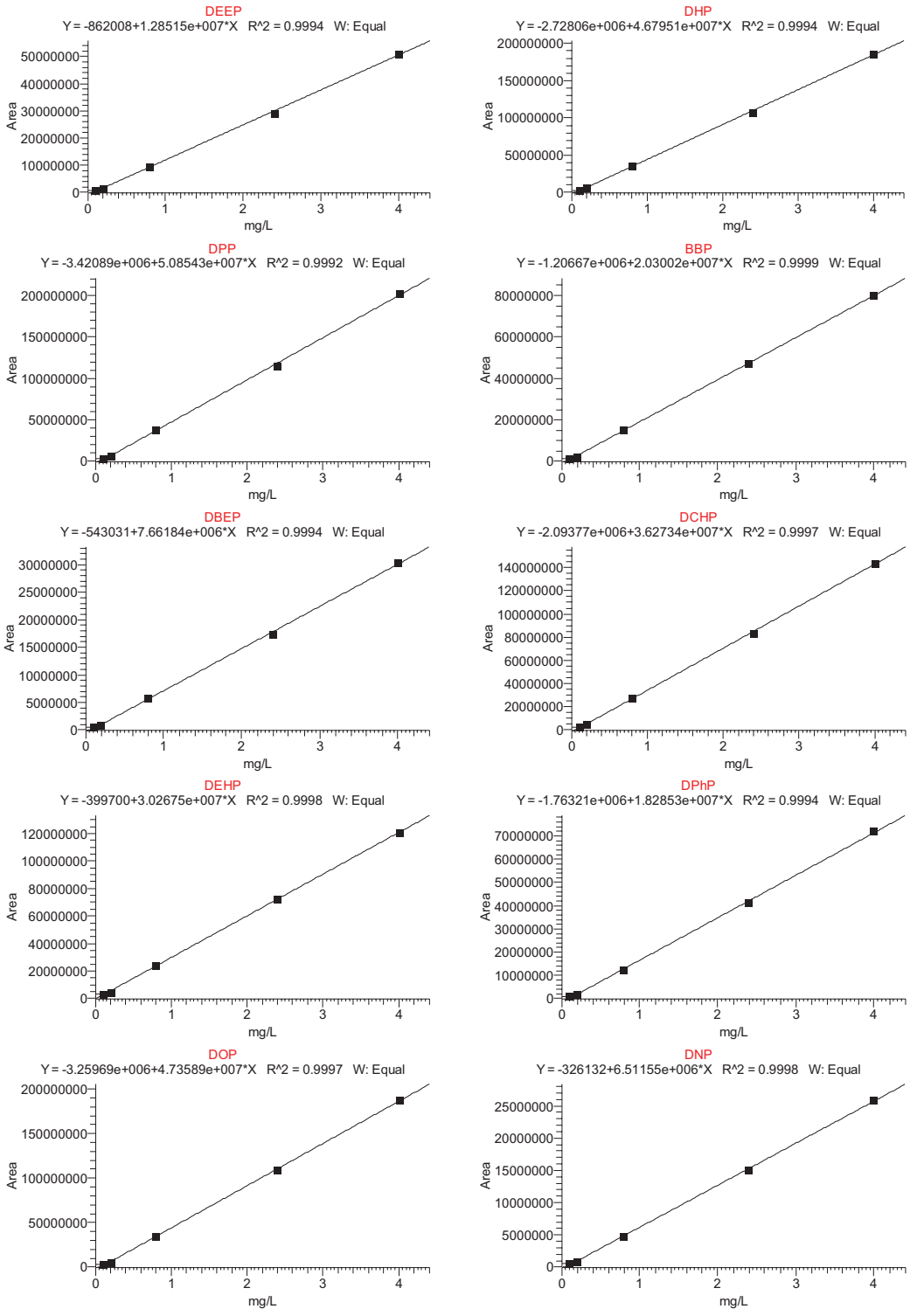


Figure 10. Calibration curves of 16 PAEs.

Table 5. Method recovery and precision data at trace level (avg. recovery 103%).

Compound name	Spike level 0.1 mg/L		Spike level 0.3 mg/L	
	Recovery %	RSD %	Recovery %	RSD %
DMP	95.0	5.4	99.0	4.7
DEP	103.0	5.5	108.0	2.2
DIBP	101.0	2.0	101.0	3.2
DBP	107.0	6.6	101.0	1.3
DMPP	105.0	3.3	107.0	5.7
DMEP	86.3	5.3	83.2	3.4
DPP	109.0	6.0	104.0	1.6
DEEP	103.0	4.1	104.0	3.2
DHP	104.0	4.6	109.0	3.7
BBP	110.0	3.6	103.0	3.7
DEHP	102.0	1.4	105.0	4.1
DBEP	104.0	5.0	108.0	4.6
DCHP	103.0	4.1	103.0	3.6
DOP	105.0	5.8	104.0	2.6
DPhP	108.0	4.2	109.0	1.8
DNP	107.0	8.4	101.0	5.4

Table 6. The phthalate ester concentration in eight commercial liquor samples(mg/L).

CompouND	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
DMP	ND	0.303	ND	ND	0.005	ND	ND	0.025
DEP	ND	ND	ND	ND	0.011	ND	ND	ND
DIBP	ND	1.526	ND	1.373	0.106	ND	ND	ND
DBP	ND	1.024	0.045	0.656	0.133	ND	0.469	0.064
DMPP	ND	ND	ND	ND	ND	ND	ND	ND
DMEP	ND	ND	ND	ND	ND	ND	ND	ND
DPP	ND	ND	ND	ND	ND	ND	ND	ND
DEEP	ND	ND	ND	ND	ND	ND	ND	ND
DHP	ND	ND	ND	ND	ND	ND	ND	ND
BBP	ND	ND	ND	ND	ND	ND	ND	ND
DEHP	0.086	0.029	0.010	0.236	0.014	0.006	0.017	0.016
DBEP	ND	ND	ND	ND	ND	ND	ND	ND
DCHP	ND	ND	ND	ND	ND	ND	ND	ND
DOP	ND	ND	ND	ND	ND	ND	ND	ND
DPhP	ND	ND	ND	ND	ND	ND	ND	ND
DNP	ND	ND	ND	ND	ND	ND	ND	ND

Note: ND = not detected

Method precision and determination of recovery at trace level

The measured liquor samples were spiked by two low concentration levels at 0.1 and 0.3 mg/L, and measured five times at each level. The results show that the average recovery even at trace level was 83.2-110%, and the relative standard deviation range (RSD, n=5) was 1.3 to 8.4%. The recovery and precision data results are shown in Table 5.

Eight samples of commercially available liquor brands were analyzed using the above described method. The concentrations of phthalate ester residues found are shown in Table 6.

The samples tested showed that DIBP, DBP, DEHP are prevalent, and DEHP was found in all the analyzed white wine samples.

Conclusions

In this study for the determination of phthalate plasticizer residues in liquor, the ISQ Series GC-MS met the special testing requirements set by the China method GB/T 21911-2008 for determining phthalates in food.

The sample preparation method for alcoholic beverages was quick and easy to accomplish. Using n-hexane as extraction solvent provided constant and high recoveries after removal of the major part of ethanol, even at trace level. The ISQ Series GC-MS measurement method is highly accurate as demonstrated with precise calibrations and spiked liquor samples.

The ISQ Series GC-MS method setup using full scan has good usability, provides the necessary high sensitivity, and delivers the complete spectrum information for identification and confirmation of a wide variety of possible phthalate ester contaminations by comparison with the NIST mass spectral library. The peak area integration on the uniquely selective PAE compound ions provided the precise, fast, and interference-free quantitative determination.

The routine quantitation of commercial samples was accomplished using TraceFinder software, which allowed the quantitation of the coeluting DNP isomers with the same high precision as the other PAE compounds under investigation.

The described determination method for phthalate plasticizers using the ISQ Series GC-MS is very sensitive and accurate. It is easy to perform, rapid, and covers a wide linear range to meet the need for trace level detection of PAEs in beverages.

References

[1] China method GB/T 21911-2008 for the "Determination of phthalates in food".

[2] Shao, Dongliang, Determination of Phthalate Ester Residues in White Spirit by GC-MS, Chemical analysis and meterage, 19(6) (2010) 33-35.

Determination of Phthalate Esters in Soft Drinks By GC-MS

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Key Words
TraceGOLD TG-5MS, phthalate esters, soft drinks, liquid-liquid extraction

Abstract
This application note demonstrates the quantitative analysis of phthalate esters in soft drinks. The combination of Thermo Scientific™ ultra low bleed TraceGOLD™ TG-5MS columns with a Thermo Scientific™ TRACE™ GC coupled with a Thermo Scientific™ ISQ™ mass spectrometer provides high sensitivity for the detection of phthalate esters in selected ion monitoring (SIM) acquisition mode.

Introduction

Phthalate esters are the main plasticizers used as softening agents in the production of PVC. These compounds are reported to act as endocrine disruptors, and exposure to high levels can cause harmful effects in the human reproductive system. There have been reports from the U. S. Food and Drug Administration that certain foods and beverages, particularly fruit juices, contain high levels of phthalates. In some cases, deliberate adulteration of soft drinks with phthalate esters has been reported.

The EU recently published a methodology without an extraction method for bis-(2-ethylhexyl)phthalate (DEHP) in sports drink at concentrations between 3000 and 100000 ng/mL [1]. In this application note, an analytical procedure for the quantitative analysis of 15 phthalate esters between the concentrations of 100 and 5000 ng/mL is reported. The extraction of the phthalate esters is based on liquid-liquid extraction. Extraction efficiencies are reported at low (300 ng/mL) and high (1000 ng/mL) concentrations in spiked drink sample.

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Experimental Details

Consumables		Part Number
Column:	TraceGOLD TG-5MS, 30 m × 0.25 mm × 0.25 µm	26098-1420
Septum:	Thermo Scientific BTO, 17 mm	31303211
Liner:	Thermo Scientific™ Splitless Straight Liner, 5 × 8 × 105 mm	45350033
Column ferrules:	100% graphite ferrules for Thermo Scientific™ TRACE™ injector, 0.1–0.25 mm i.d.	29053488
Colum ferrules:	Graphite/Vespel® for transfer line 0.1–0.25 mm i.d.	29033496
Injection syringe:	10 µL fixed needle syringe for a Thermo Scientific™ TriPlus™ Autosampler	36500525

Sample Handling Equipment		Part Number
System:	Thermo Scientific™ eVol™ Sample Dispensing System (containing Sample Dispensing System, eVol XCHANGE™ Syringes in 5, 50, and 500 µL volumes and eVol stand)	66002-024
Vials and closures:	Thermo Scientific 9 mm Wide Opening Screw Thread Vial Convenience Kit, 2 mL Clear Vial with Patch, Blue Polypropylene Closure with Clear PTFE/Blue Silicone Septa	60180-599

Chemicals and Reagents	Part Number
Fisher Scientific™ HPLC grade acetone	A/0600/15
Fisher Scientific HPLC grade dichloromethane	D/1856/17
Fisher Scientific HPLC grade hexane	H/0406/15
Fisher Scientific HPLC grade water	W/0106/17

Preparation of Calibration Standard

A stock standard solution of 1000 µg/mL of phthalate esters listed in Table 1 was prepared in hexane / acetone (8:2 v/v) (Standard A). This was then used to prepare standard solutions in hexane of 5000, 3000, 1000, 500, 300, and 100 ng/mL. These were Standards B–G. For construction of the calibration curve, the standards B–G were fortified with benzyl benzoate as an internal standard to a level of 1000 ng/mL.

Sample Preparation

The standards and extracts were prepared in scrupulously clean glassware. Avoiding any contact with plastic is vital as phthalates can contaminate glassware and blank samples very easily. Glass analytical syringes, glass pipettes, and pesticide grade solvents were used for preparing samples.

Glassware was scrupulously cleaned by rinsing first with water and then with acetone and hexane. For accuracy, the calibration samples were prepared using an eVol dispensing system. This avoided the use of plastic pipettes as the eVol system has a glass syringe barrel and stainless steel dispensing needle. Any plastic containers were avoided to reduce potential phthalate contamination in the sample preparation.

To prepare the spiked soft drink samples, 300 ng/mL and 1000 ng/mL of phthalate esters (listed in Table 2) prepared in acetone were spiked into 5 mL of soft drink followed by the addition of 5 mL of dichloromethane containing 1000 ng/mL internal standard. The solution mixture was shaken vigorously. An aliquot of organic layer was transferred to the GC vial.

A high injector temperature (320 °C) was used to release the high MW phthalates or any adsorbing phthalates in the injector head. The high temperature resistant BTO septa were used.

Separation Conditions	
Instrumentation:	Thermo Scientific™ TRACE™ GC Ultra gas chromatograph
Carrier gas:	Helium
Column flow:	1.0 mL/min, constant flow
Oven temperature:	80 °C (1 min), 10 °C/min, 320 °C (8 min)
Injector type:	Split/Splitless
Injector mode:	Splitless (1 min), 20 mL/min flow rate, constant septum purge
Injector temperature:	320 °C

MS Conditions	
Instrumentation:	Thermo Scientific™ ISQ™ GC single quadrupole mass spectrometer
Transfer line temperature:	300 °C
Source temperature:	260 °C
Ionization conditions:	EI
Electron energy:	70 eV
SIM scan parameters:	Table 1

Compound	Abbrev	Scan start time (min)	Quan ion (Qual Ion)	Dwell time (s)
Dimethylphthalate	DMP	7.0	163 (194, 77)	0.08
Diethylphthalate	DEP	10.50	149 (177, 121)	0.08
Benzyl benzoate (Internal standard)	ISTD	12.50	105 (212, 91)	0.08
Diisobutyl ester phthalic acid	DIBP	14.00	149 (223, 205)	0.08
Di-n-butyl phthalate	DBP	15.00	149 (223, 205)	0.08
Bis(2-methoxyethyl) phthalate	DMEP	15.75	59 (149,193)	0.08
Bis(4-methyl-2-pentyl) phthalate	BMPP	16.30	149 (251,167)	0.08
Bis(2-ethoxyethyl) phthalate	DEEP	16.80	45 72,149	0.08
Dipentylphthalate	DPP	17.20	149 (237, 219)	0.08
Di-n-hexyl phthalate	DHXP	18.20	104 (149, 76)	0.04
Benzyl butyl phthalate	BBP	18.20	149 (91, 206)	0.04
Bis(2-n-butoxyethyl) phthalate	DBEP	19.50	149 (223, 205)	0.08
Dicyclohexylester Phthalic acid	DCHP	20.30	149 (167, 83)	0.08
Dioctyl Bis(2-ethylhexyl) phthalate	DEHP	20.30	149 (167, 279)	0.08
Di-n-octyl phthalate	DNOP	21.40	149 (279, 167)	0.08
Di-nonyl Phthalate	DNP	22.80	149 (293, 71)	0.08

Table 1: SIM Scan parameters

Injection Conditions	
Instrumentation:	TriPlus Autosampler
Injection volume:	1 µL
Pre- and post-injection dwell time:	3 s

Data Processing	
Software:	Thermo Scientific™ Xcalibur™ software

Results

The analysis was carried out using a TraceGOLD TG-5MS column. The chromatogram of 1000 ng/mL of spiked phthalate ester in soft drink, in a full scan mode, is shown in Figure 1.

Phthalate esters are environmentally ubiquitous and this may therefore affect measured recoveries. To minimize this problem, care was taken when preparing solutions for calibration and extractions. Longer chain phthalate esters such as DNP, DNOP, and DHXP can absorb to the glassware and the lower extraction recoveries were expected.

The results for the calibration data and for the phthalate esters extracted from a soft drink are displayed in Table 2. The coefficients of determination (R^2) between the area ratio of sample and internal standard for all phthalate esters were > 0.99 (Table 2), demonstrating excellent method linearity using the TraceGOLD TG-5MS GC column.

The extraction of phthalate esters at 300 ng/mL and 1000 ng/mL gave recoveries between 66–111% and 71–118%, respectively.

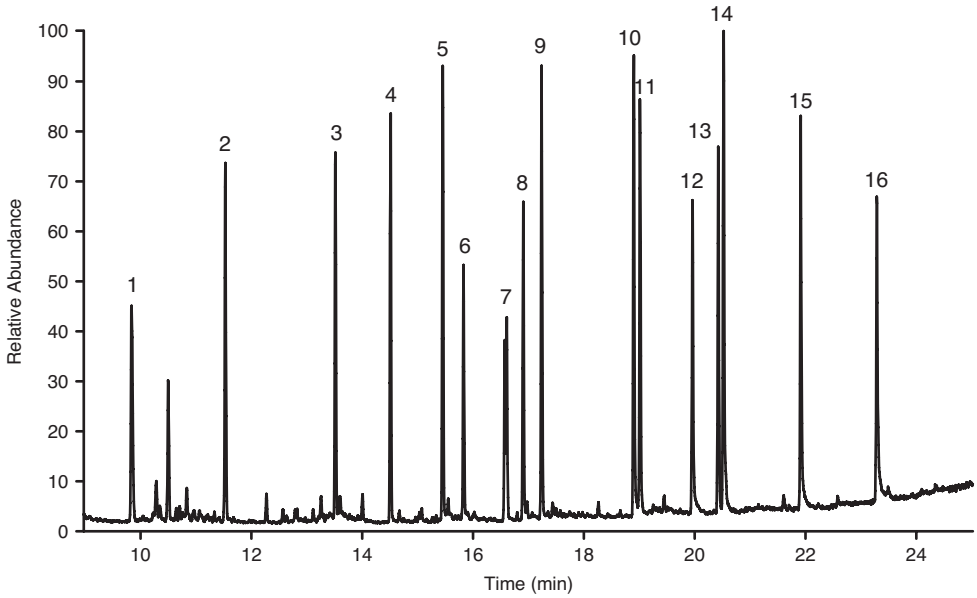

Figure 1: TIC of 1000 ng/mL phthalate esters standard in full scan 40–450 amu

Peak	Compound	Abbrev	t_R (min)	R^2	% Recovery at 300 ng/mL (n=3)	% Recovery at 1000 ng/mL (n=2)
1	**Dimethylphthalate**	DMP	9.91	0.9944	103	85
2	**Diethylphthalate**	DEP	11.60	0.9953	106	108
3	**Benzyl benzoate (Internal standard)**	ISTD	13.60			
4	**Diisobutyl ester phthalic acid**	DIBP	14.58	0.9970	92	118
5	**Di-n-butyl phthalate**	DBP	15.52	0.9964	91	92
6	**Bis(2-methoxyethyl) phthalate**	DMEP	15.90	0.9918	93	96
7	**Bis(4-methyl-2-pentyl) phthalate**	BMPP	16.6, 16.68	0.9975	95	82
8	**Bis(2-ethoxyethyl) phthalate**	DEEP	16.98	0.9913	100	93
9	**Dipentylphthalate**	DPP	17.31	0.9940	107	87
10	**Di-n-hexyl phthalate**	DHXP	18.98	0.9945	84	71
11	**Benzyl butyl phthalate**	BBP	19.09	0.9937	111	86
12	**Bis(2-n-butoxyethyl) phthalate**	DBEP	20.03	0.9930	77	77
13	**Dicyclohexylester Phthalic acid**	DCHP	20.50	0.9925	107	84
14	**Dioctyl Bis(2-ethylhexyl) phthalate**	DEHP	20.60	0.9941	100	78
15	**Di-n-octyl phthalate**	DNOP	21.99	0.9962	71	80
16	**Di-nonyl Phthalate**	DNP	23.37	0.9947	66	79

Table 2: Recoveries and linearity of phthalate esters according to their retention times

Conclusion

A method for the quantification of phthalate esters in soft drinks has been developed on a TraceGOLD TG-5MS column. The ultra low bleed characteristics of the column enable the detection of low levels of phthalate esters using the ISQ mass spectrometer in SIM mode.

References

[1] EU commission JRC technical notes: Determination of bis-(2-ethylhexyl)phthalate (DEHP) in sports drinks by isotopic dilution headspace solid-phase micro extraction gas chromatography mass spectrometry (HS-SPME-GC-MS).

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Application Notebook

Additional Contaminants

Other Contaminants

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Application Note 10315

High Sensitivity Analysis of Nitrosamines Using GC-MS/MS

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Keywords: Nitrosamines, Food Safety, beer, TSQ 8000, GC-MS/MS, quantitation, confirmation, AutoSRM, TraceFinder

Introduction

Nitrosamines is the common term used for compounds of the class of N-nitrosodialkylamines. A large variety of compounds are known and described with different alkyl moieties [1]. The simplest N-nitrosodialkylamine with two methyl groups is the N-nitrosodimethylamine (NDMA). Nitrosamines are in common highly toxic compounds with high cancerogenity for humans and animals, in higher doses leading to severe liver damage with internal bleeding [2,3].

Nitrosamines in food are mainly produced from nitrites. Nitrites are added to food as preservatives in meat and meat products preventing the Botulinus poisoning. Antioxidant food additives like vitamin C can prevent the formation of nitrosamines from nitrites [4]. Another source of nitrosamines is described by the reaction of nitrogen oxides with alkaloids as it is reported from the drying process of the germinated malt in beer production [5]. As nitrosamine levels in malt and beer have been significantly reduced in the brewing process, high analytical performance is required. In addition to the regular control of other food products for daily consumption, malt in beer is also monitored for low levels of nitrosamines.

The “classical” nitrosamine analysis was performed for many years by gas chromatography using a thermal energy analyzer (TEA) as detector. This special TEA detector was used due to its selectivity for nitrosamines with to the specific chemilumniscent reaction of ozone with the detector generated NO from nitrosamines. Today, with increased sensitivity requirements, the detection limits of the TEA, and also its complex operation, no longer comply with the required needs for low detection limits and sample throughput. Mass spectrometric methods have increasingly replaced the TEA.

The EPA method 521 by Munch and Bassett from 2004 provided at that time a suitable GC-MS method based on chemical ionization (CI) using an ion trap mass spectrometer with internal ionization [6, 7], in contrast

to standard quadrupole or ion trap mass spectrometers using a dedicated (external) ion source design. Current developments in GC-MS triple quadrupole technology deliver today very high sensitivity and selectivity also in the small molecule mass range and allow the detection of nitrosamines at very low concentration levels even in complex matrix samples. This is made possible by using a much simpler and standard approach with the regular electron impact ionization (EI) for a very straightforward method for low level nitrosamine analysis.

This application note describes a turn-key GC-MS/MS method for routine detection and quantitation of food borne nitrosamine compounds. The food matrix in this work has been different malt beer products and as a final food product the commercial beer. Special focus in the method development has been made to provide the required high sensitivity for the detection of the nitrosamine compounds for a fast, easy to implement routine method.

The sample preparation is adapted and slightly modified from AOAC Official Method (2000), 982.11 [8]. An SPE column extraction method using a celite column and elution with DCM to isolate the nitrosamines from the beer samples was developed.

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Experimental Conditions
GC-MS/MS Instrument

TRACE 1310 GC

iC Injector Module	Split/Splitless Injector
Injector Temperature	250 °C
Injection mode	splitless
Surge mode	300 KPa
Splitless Time	1.0 min
Analytical Column	TG-WAX MS, 30m×0.25mm×0.5µm
Carrier gas	He （99.999% purity）
Flow rate	1.0 mL/min, constant flow
Oven Program	45 °C for 3 min,
	25 °C/min to 130 °C,
	12 °C/min to 230°C, 1min hold
Transfer line Temperature	250°C
Total analysis time	14.7 min
Total cycle time	18.4 min

TriPlus RSH Autosampler

Injection Volume	1 µL
Solvent	dichloromethane
Standard runs	3 replicate of injections each
Dilution of standard mix	1ppb, 5ppb, 10ppb, 25ppb, 100ppb, 250ppb, 500ppb
Internal standard	NDPA added to each calibration level at 50ppb

TSQ 8000 Triple Quadrupole GC-MS/MS system

Ionization mode	EI
Mass resolution setting	normal
Source temperature	220 °C
Scan mode	MRM, retention time-based SRM mode

MRM Method Setup

The triple quadrupole MS method setup was performed by using the AutoSRM software which is part of the Thermo Scientific TSQ™ 8000 GC-MS/MS software suite. The method generated by AutoSRM was used without any additional manually modification. One autosampler vial containing a standard solution of the nitrosamine compounds to be analyzed has been used only for the AutoSRM process.

The AutoSRM procedure automatically runs the following three steps:

1. First a full scan analysis of the standard solution (Figure 1). Get the most intense ions of the full scan spectra to be used as the precursor ions.
2. Run a next analysis acquiring the product ion spectra from the selected precursor ions (the number of precursor ions to be used can be configured to the analytical needs). Get the most intense product ions from each precursor ion (optionally the desired precursor ions can be selected manually for further optimization).

Table 1. MRM method setup using AutoSRM

	Precursor	Product	Collision Energy (eV)	Retention Time (min)	Time Window (min)
NDMA	74	42.1	15	7.89	1
	74	43.8	5	7.89	1
NDEA	102	44.1	10	8.56	1
	102	85.1	5	8.56	1
NDPA (ISTD)	130	42.9	10	9.76	1
	130	113.1	5	9.76	1
NDBA	158	99.1	5	11.35	1
	158	141.1	5	11.35	1
NPIP	114	41.5	15	11.80	1
	114	83.9	5	11.80	1
NPYR	100	43	10	12.06	1
	100	55.1	5	12.06	1
NMOR	116	56.1	10	12.47	1
	116	86.1	5	12.47	1

** The transitions marked as grey color are quantitation ions*

3. Optimize for all compounds the collision energy of the selected precursor/product ion transitions for maximized compound response and best method sensitivity (Figure 2).

Initiated by the AutoSRM procedure as many as necessary autosampler injections from the one standard vial are scheduled.

As a result of the AutoSRM program, the generated SRM transition table shown in Table 1 has been automatically built. The table represents at the same time the TSQ 8000 GC-MS/MS system MRM acquisition method using the timed-SRM mode with a short acquisition window of 60 s around the compound retention time. No other setting of scan segments is necessary, or will be necessary in case additional compounds need to be added to the acquisition, other than the compound retention time.

Sample Measurements

From the large variety of potential nitrosamines the compounds that had been included in this method are those that are reported to be of relevance in the germinated malt drying process. Samples analyzed included malt beer as unspiked samples and 4% ethanol as sample blanks. In case of the analysis of other food matrices, additional compounds can be added to this method easily at any time as described in the method setup by AutoSRM [9, 10].

Results

The chromatograms of the nitrosamines included in this method show a quick elution of the compounds from 7.87 min NDMA to 12:47 min allowing a short cycle time for increased sample throughput. The peak intensities are retained in Figure 3 at the lowest calibration level of 1 ppb. NDMA can be detected with good S/N values.

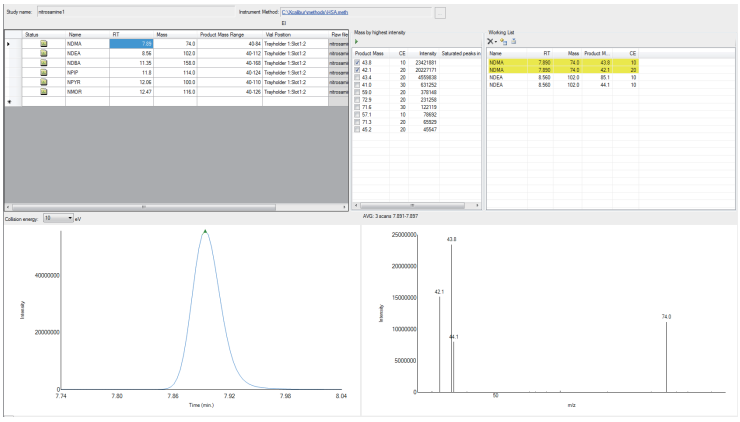

Figure 1. AutoSRM Precursor Ion Selection for NDMA from EI full scan spectra

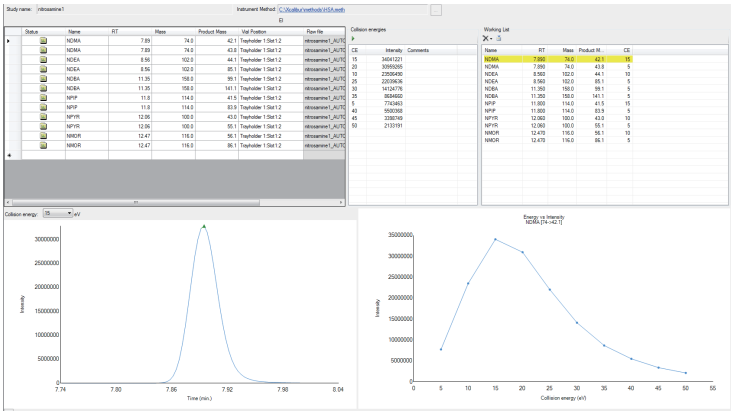

Figure 2. AutoSRM collision energy optimization for all nitrosmaine precursor ions

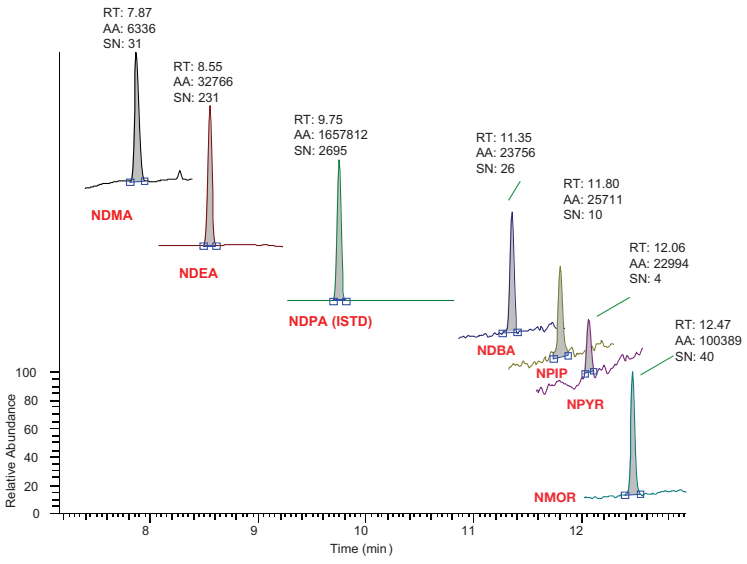

Figure 3. Chromatogram of the standard mix at 1 ppb

The quantitative calibration has been performed in a wide concentration range from 1 ppb to 500 ppb. Figure 4 shows the chromatogram peaks of NDMA from all the calibration runs. In all cases the NDMA peak shape is perfectly symmetrical, no tailing occurs and the peak area integration provides very reliable values without the need for any further manual corrections. The linear calibration of NDMA used to quantify the samples is shown in Figure 5 with very good correlation of R^2 better than 0.99. The same good calibration precision is achieved for all nitrosamines in this TSQ 8000 GC-MS/MS method.

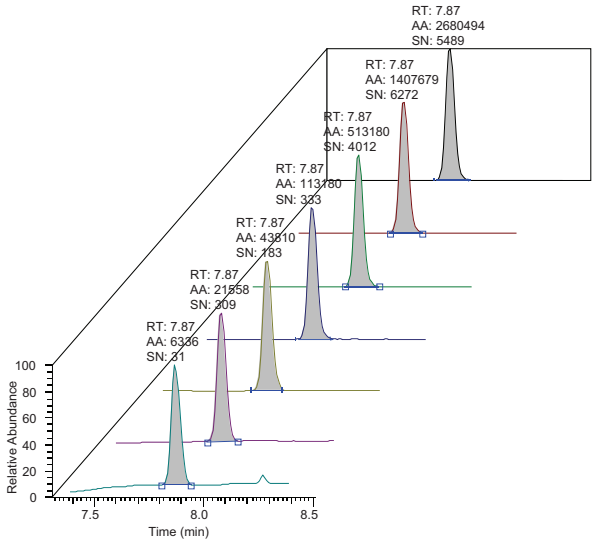

Figure 4. NDMA calibration runs from 1 ppb (bottom) to 500 ppb (top)

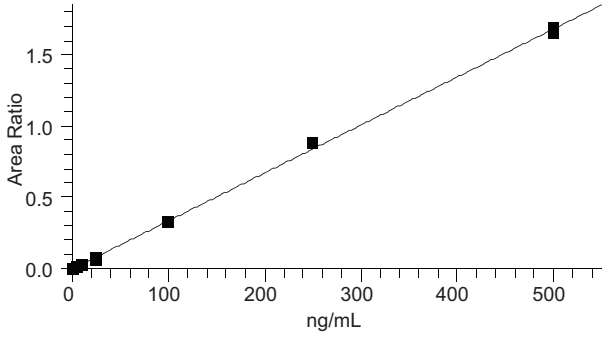

Figure 5. Linear calibration function for NMDA from 1 ppb to 500 ppb

LOQ Determination

The calculation of the LOQ and LOD was based on the S/N achieved for a chromatographic peak.The LOQ calculation is based on the level of S/N 10, and LOD values are calculated based on a S/N of 3.

Table 2. Calculation of the method LOQ and LOD

Compound	S/N @ 1ppb	Calculated LOQ (ppb)	Calculated LOD (ppb)
NDMA	13	1.0	0.25
NDEA	231	0.05	0.02
NDBA	23	0.5	0.20
NPIP	10	1.0	0.50
NMOR	40	0.3	0.10
NPYR	24	3	1.0

Confirmation

For compound confirmation the ion ratio check provided by the Thermo Scientific TraceFinder™ quantitation software was used by comparing the ion intensity of the second acquired SRM transition with the first SRM used for quantitation. The precision for the ion ratio was calculated using the three replicate standard runs over the complete concentration range from 1 ppb to 500 ppb and is shown in Table 3. Although the detected ions all are in the low mass range and potentially subject to many interferences the precision of the product ion ratio is very good in the range of 1-4%.

For quality control purposes in sample analyses the confirmation of a positive result is done by the ion ratio check during the quantitation data processing in TraceFinder software. The ion ratio of the two acquired product ions is required to stay within +/- 5% (10%) for all compounds, compared to the calibrated value from the standard runs. This provides a solid safety margin for routine sample measurements. Table 3 indicates the used average value (AVG) of the ion ratio for all nitrosamines investigated.

Sample Measurements

A number of samples have been measured, including blanks and spiked beer samples. The results of a blank sample are shown in Table 4. The found low NDMA concentration in this sample has been calculated below the calibration, and also below LOQ. The blank sample could be confirmed to be free from nitrosamine compounds at the given LOQ.

Another sample was prepared from beer that has been spiked with different amounts of nitrosamines. All nitrosamine compounds have been detected and quantified in a low concentration range of 9 – 13 ppb, see Table 5. Each quantified peak passed the ion ratio quality control and could be positively confirmed at this low level by calculating the product ion ratios for each of the compounds.

Conclusions

With the described GC-MS/MS method on the TSQ 8000 system all nitrosamine compounds under investigation could be safely detected and precisely quantified at the required low levels for a safe food control.

The LODs of all compounds have been determined to be below 1 ppb, using 1 ppb as the lowest concentration for the quantitative calibration.

The TSQ 8000 GC-MS/MS shows a wide linearity in the range of 1-500 ppb with very good precision. All calibration curves have been shown to be strictly linear with R^2 better than 0.99.

The TSQ 8000 GC-MS/MS shows great ion ratio stability for the confirmation of positive samples. The RSD% of the ion ratio of all compounds is lower than 4% even at LOQ level.

The use, setup and maintenance of a GC-MS/MS method for nitrosamines is easy. The unique AutoSRM software finds and optimizes the SRM transitions and collision energy automatically, even facing new and yet unknown components.

Based on the demonstrated GC-MS/MS method, the TSQ 8000 GC-MS/MS can successfully quantify the concentration of nitrosamine components in real samples without any uncertainty.

The described GC-MS/MS method for food nitrosamines on the TSQ 8000 GC-MS/MS can serve as a turnkey method for routine use in food safety control. It is using standard GC-MS/MS triple quadrupole instrumentation which is also common for many other areas of regular food safety control, e.g. pesticides, POPs or polyaromatic hydrocarbons. The presented method is fast, allows high sample throughput, and provides results with very high sensitivity and precision. With this standard EI ionization method setup this presented method for low level nitrosamine quantitation is recommended to be employed as a productive alternative to the earlier described chemical ionization ion trap procedure using liquid CI reagents.

Table 3. Precision of the confirming ion ratios from 1 ppb – 500 ppb

Concentration (ppb)	1	5	10	25	100	250	500	AVG	RSD (%)
NDMA	70.7	67.9	68.0	69.8	69.1	71.9	69.6	69.6	2.01
NDEA	20.8	22.1	22.5	22.4	22.5	22.5	22.5	22.2	2.84
NDBA	102.4	102.4	98.2	98.6	96.1	93.4	99.2	98.6	3.28
NPIP	6.1	5.5	6.2	5.9	6.0	6.1	6.2	6.0	3.88
NPYR	-	64.6	62.4	66.2	66.9	68.1	66.7	65.8	3.06

Table 4. Results of a blank sample

Compound	Area	ISTD Area	Area Ratio	Ion Ratio Confirmation	Calculated Amount (ppb)
NDMA	2591.368	2028129.842	0.001	Pass (65.1%)	0.74*
NDEA	1875.386	2028129.842	0.001	Fail (0%)	N/A
NDBA	6806.996	2028129.842	0.003	Fail (81.1%)	N/A
NPIP	N/A	2028129.842	N/A	N/A	N/A
NPYR	N/A	2028129.842	N/A	N/A	N/A
NMOR	4415.782	2028129.842	0.002	Fail (0%)	N/A

**Below LOQ*

Table 5. Results from a spiked beer sample

Compound	Area	ISTD Area	Area Ratio	Ion Rato	Calculated Amount (ppb)
NDMA	91318.135	2282168.009	0.040	Pass (68.3%)	12.0
NDEA	480955.478	2282168.009	0.211	Pass (22.0%	9.4
NDBA	402754.561	2282168.009	0.176	Pass (96.8%)	13.2
NPIP	280162.125	2282168.009	0.123	Pass (5.9%)	10.1
NPYR	318081.273	2282168.009	0.139	Pass (68.9%)	13.3
NMOR	1145719.054	2282168.009	0.502	Pass (67.9%)	10.1

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Method: 51980A

Determination of Polycyclic Aromatic Hydrocarbons (PAHs) and Aliphatic Hydrocarbons in Oysters by GC-MS/MS

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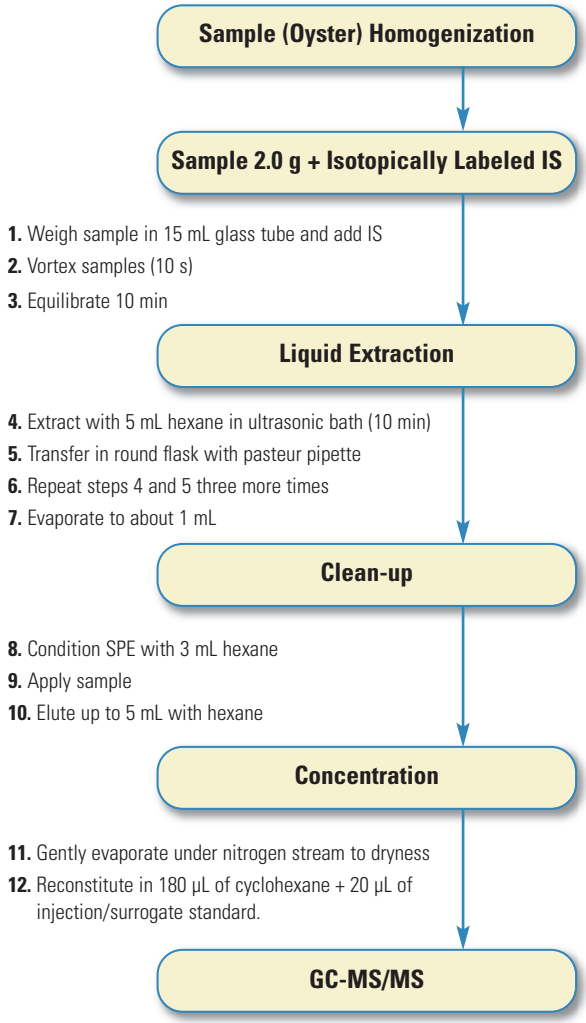
Key Words

- TSQ Quantum XLS
- Aliphatic Hydrocarbons
- Gulf Oil Spill
- Oil Contamination
- Oyster Extraction
- PAHs

1. Schematic of Method



2. Scope

This method can be applied to oysters to detect the presence of aliphatic hydrocarbons and PAH contamination from crude oil found in the Gulf of Mexico in late May 2010. From the profile using GC-MS/MS, the method can be used to characterize the source of contamination. The method can give a semi-quantitative indication of whether levels of PAHs exceed safety limits for human consumption of oysters.

3. Principle

The method uses a liquid extraction of oysters with hexane, followed by a clean-up on a silica-SPE-cartridge. The sample is fortified with appropriate labeled internal standards and analyzed by simultaneous GC-MS/MS using a

Thermo Scientific TSQ Quantum XLS triple quadrupole mass spectrometer system. Aliphatic hydrocarbons and PAHs of food safety significance are measured and compared with the profile from crude oil collected from the Gulf of Mexico in late May 2010.

4. Reagent List

		Fisher Scientific USA Part Number
4.1	Acetone	A9491
4.2	Cyclohexane	C6201
4.3	Hexane	H3021
4.4	SPE Hypersep SI, 200 mg/3 mL	03251270
4.5	Toluene	AC176850010

5. Calibration Standards

5.1 PAHs

Acenaphthene – Ace (Sigma)
Acenaphthylene – Acy (Sigma)
Anthracene – Ant (Sigma)
Benz[a]anthracene – B(a)A (Sigma)
Benzo[a]pyrene – B(a)P (Sigma)
Benzo[b]fluoranthene – B(b)F (Sigma)
Benzo[g,h,i]perylene – B(g,h,i)P (Sigma)
Benzo[k]fluoranthene – B(k)F (Sigma)
Chrysene – Chr (Sigma)
Dibenz[a,h]anthracene – D(a,h)A (Sigma)
Fluoranthene – Flu (Sigma)
Fluorene – Fln (Sigma)
Indeno[1,2,3-cd]pyrene – I(1,2,3-c,d)P (Sigma)
Naphthalene – Naph (Sigma)
Phenanthrene – Phe (Sigma)
Pyrene – Pyr (Sigma)

5.2 Injection Standard

5-methylchrysene – 5-MChr (Dr. Ehrenstorfer)

5.3 Internal Standards

Anthracene-D10 – Ant-D10 (Sigma)
Benzo[a]pyrene-D12 – B(a)P-D12 (Sigma)
Benzo[ghi]perylene-D12 – B(g,h,i)P-D12 (LGC Standards)
Chrysene-D12 – Chr-D12 (Sigma)

5.4 Quality Control Materials

Petroleum Crude oil (NIST Standard Reference Material®, 1582)

Aliphatic Hydrocarbons in 2,2,4-Trimethylpentane (NIST Standard Reference Material, 1494)

6. Standards and Reagent Preparation

6.1 Stock solutions of 2 µg/mL of PAH standards in toluene

6.2 Internal PAHs standard (IS) concentration: 2 µg/mL (Benzo[ghi]perylene-d_{12}, Anthracene-d_{10}, Chrysene-d_{12}) in toluene and 200 µg/mL Benzo[a]pyrene-d_{12} in cyclohexane

6.3 Working standard solution mixture of 16 PAHs in toluene (100 ng/mL)

6.4 Working internal standard mixture of IS PAHs in toluene (200 ng/mL)

6.5 Syringe standard, 5-methyl-chrysene (200 ng/mL) in toluene.

6.6 Spiked solution of Petroleum crude oil (NIST 1582): 100 mg/mL in cyclohexane

7. Apparatus

		Fisher Scientific USA Part Number
7.1	Centrifuge, Heraeus™ Multifuge™ X3	75-004-500
7.2	Thermo Scientific 16 port SPE vacuum manifold	03-251-252
7.3	Evaporator EVTM-130-32-16 (Fisher Scientific Germany)	3106395
7.4	Fisher precision balance	01918306
7.5	Vacuum pump	05-402-100
7.6	Rotavapor® R-210	05-024-21
7.7	Sartorius analytical balance	01-910-3224
7.8	Thermo sci. Barnstead EASYpure™ II water	0905050
7.9	Ultrasonic bath Elmsonic S40H	154606Q
7.10	ULTRA-TURRAX® – dispergation tool	1425980
7.11	ULTRA-TURRAX – Plug-in coupling	14259023
7.12	ULTRA-TURRAX	142259301
7.13	Vortex shaker	14505141
7.14	Vortex standard cap	14-505-140
7.15	GC column TR-50MS 30 m, 0.25 mm ID, 0.25 µm film	260R142P
7.16	TSQ Quantum XLS™ Triple Quadrupole Mass Spectrometer	

8. Consumables

		Part Number
8.1	GC vials	03393F
8.2	Pipette Finnpipette 100-1000 µL	14386320
8.3	Pipette Finnpipette 10-100 µL	14386318
8.4	Pipette Finnpipette 500-5000 µL	14386321
8.5	Pipette holder	14245160
8.6	Pipette Pasteur soda lime glass 150 mm	136786A
8.7	Pipette suction device	03-692-350
8.8	Pipette tips 0.5 – 250 µL, 500/box	21377144
8.9	Pipette tips 1 – 5 mL, 75/box	2137750
8.10	Pipette tips 100 – 1000 µL, 200/box	2137746
8.11	Spatula, 18/10 steel	14356C
8.12	Spatula, nylon	NC9319088
8.13	SPE Hypersep SI, 200 mg/3 mL, 50 pc.	03251270
8.14	Tube holder	03840233
8.15	Wash bottle, PTFE	0340911A

Glassware

8.16	Beaker, 50 mL	FB10050
8.17	Fisherbrand test tubes	14-958D
8.18	Funnel, 55 mm	14353D
8.19	Glass tubes	14957E
8.20	Pasteur pipette	136786A
8.21	Round flask 50 mL, NS 29/32 (Fisher Scientific Germany)	9011835
8.22	Volumetric flask, 10 mL	FB40110
8.23	Volumetric flask, 25 mL	10200A

9. Procedure

9.1 Sample Preparation

Rinse the glassware with acetone before proceeding with the method to avoid cross contamination. Homogenize a suitable amount (e.g. 250 g) of oyster meat appropriately to give a slurry using a high speed blender, e.g. ULTRA-TURRAX.

9.2 Extraction

9.2.1 Accurately weigh the homogenized sample (ca. 2 g) into a glass tube.

9.2.2 Add 50 µL of PAH internal standard solution to the sample.

9.2.3 Vortex the mixture for 10 s and wait 10 min for equilibration.

9.2.4 Add 5 mL of hexane to the sample and put it into an ultrasonic bath for 10 min.

9.2.5 Transfer the supernatant hexane layer into a 50 mL round flask with a Pasteur pipette.

9.2.6 Repeat the extraction (9.2.4 and 9.2.5) three more times.

9.2.7 Centrifuge for 5 min at 4500 rpm and 5 °C and decant supernatant.

9.2.8 Evaporate to 1 mL under vacuum (220 mbar/50 °C).

9.3 Clean-up

9.3.1 Condition the SPE-Cartridge with 3 mL of hexane.

9.3.2 Apply the extract to the cartridge and elute into an evaporator tube with 5 mL of hexane.

9.3.3 Evaporate at 40 °C to dryness using a blow-down apparatus under a gentle stream of nitrogen.

9.3.4 Reconstitute in 180 µL of cyclohexane plus 20 µL of injection standard.

9.4 Analysis

9.4.1 GC operating conditions

GC analysis was performed on a Thermo Scientific TRACE GC Ultra system (Thermo Fisher Scientific, Waltham, MA USA). The GC conditions were as follows:

Column: Thermo TR-50MS 30 m, I.D.: 0.25 mm, 0.25 µm film capillary column
Injection mode: splitless with a 5 mm injection port liner
Injection port temperature: 270 °C
Flow rate: 1.2 mL/min
Split flow: "On", flow: 25 mL/min
Splitless time: 1 min
SSL carrier method mode: constant flow
Initial value: "On" with 1.2 mL/min
Initial time: 1 min
Gas saver flow: 15 mL/min
Gas saver time: 3 min
Vacuum compensation: "On"
Transfer line temperature: 270 °C
Oven Temperature: 60 °C for 1 min, then programmed at 12 °C/min to 210 °C, then 8 °C/min to 340 °C with 5 min hold time

9.4.2 Mass Spectrometric Conditions

MS analysis is carried out using a TSQ Quantum XLS triple quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA USA). A satisfactory tune of the mass spectrometer is achieved when the detector is set at *m/z* 300 or less and the three FC 43 (calibration gas) ions (68, 219, and 502) are at least half the height of their respective windows and the ions at 502 and 503 are resolved.

The MS conditions for PAHs are as follows:

Ionization mode: EI positive ion
Ion volume: closed EI
Emission current: 50 uA
Ion source temperature: 250 °C
Scan type: Full scan in range *m/z* 45-650 and SRM
Scan width: 0.15 for SRM
Scan time 0.2 s for full scan and 0.05 for SRM
Peak width: Q1, 0.7 Da; Q3, 0.7 Da FWHM
Collision gas (Ar) pressure: 0.5 mTorr

The mass spectrometer is programmed to be able to simultaneously monitor the hydrocarbon profile in scanning Full Scan (FS) GC-MS *and* quantify the presence of PAHs by MS/MS within a single chromatographic run. Eight segments are programmed each with 2 simultaneous scan events. One scan event is used to monitor the aliphatic hydrocarbon profile throughout the whole chromatographic run (i.e in all segments), while SRM traces are set up for the target PAHs in the other scan event. The program of segments for SRM events (#1) is shown in Table 1.

Setting of scan event #2 for hydrocarbon profiling was kept constant in all segments:

- Scan type: FS in range 45-650 *m/z*
- Scan time: 0.2 s
- FWHM: 0.7 Da
- Collision gas pressure: 0.5

10. Calculation of Results

10.1 Aliphatic Hydrocarbons

From the scanned GC-MS data, print a reconstructed ion chromatogram (extracted ion chromatogram) for *m/z* 57 and plot this alongside a similar *m/z* 57 extracted chromatogram for the standard mixture of hydrocarbons. Any detectable aliphatic hydrocarbon peaks in oysters can be identified based on their retention times which are given in Table 2. This is illustrated in Figure 1. Measure the specific peak area ratios to characterize the source of hydrocarbon contamination.

10.2 PAHs

The occurrence of one or more of any of the 16 PAHs of food safety concern is indicated by the presence of transition ions (quantifier and qualifier) as indicated in Table 1 at retention times corresponding to those of the respective standards shown in Table 1. This is illustrated in Figure 1. Careful visual inspection of the SRM chromatograms should be carried out to check for interferences. The measured peak area ratios of precursor to quantifier ion should be in close agreement with those of the standards as shown in Table 1. If the presence of any of the 16 PAHs is confirmed based on retention times and ion ratios then quantification should be carried out as indicated below.

Calibration by the internal standardization is applied for the quantification of PAHs. This calibration requires the determination of response factors R_f defined by the equation below.

Calculation of the response factor:

$$R_f = \frac{A_{St} \times c_{[IS]}}{A_{[IS]} \times c_{St}}$$

$\mathbf{R_f}$ – the response factor determined by the analysis of standards PAH and internal standard

$\mathbf{A_{St}}$ – the area of the PAH peak in the calibration standard

$\mathbf{A_{[IS]}}$ – the area of the internal standard peak for the calibration standard

$\mathbf{c_{St}}$ – PAH concentration for the calibration standard solution

$\mathbf{c_{[IS]}}$ – the internal standard concentration for the calibration standard solution

Calculations for each sample the absolute amount of PAH that was extracted from the sample:

$$X_{PAH} = \frac{A_{PAH} \times X_{[IS]}}{A_{[IS]S} \times R_f}$$

X_{PAH} – the absolute amount of PAH that was extracted from the sample

A_{PAH} – the area of PAH peak of the sample
$A_{[IS]S}$ – the area of the internal standard peak of the sample
$X_{[IS]}$ – the absolute amount of internal standard added to the sample

The concentration of PAH in the sample (ng/g):

$$c\ (ng/g) = \frac{X_{PAH}}{m}$$

c – the concentration of PAH in the sample (ng/g)
m – the sample weight in g

11. Interpretation of Results

The analytical data generated in the method requires careful interpretation to collect convincing evidence of aliphatic hydrocarbon contamination of oysters originating from an actual crude oil sample from Gulf of Mexico and consequent PAH contamination. The method provides a hydrocarbon profile and PAH profile which can be matched against that of crude oil sample from the Gulf of Mexico. The composition of crude oil from the Gulf of Mexico is given in Table 4 indicating relatively high levels of n-hexadecane, n-heptadecane and pristane which are characteristic. Characteristic pristane/C-17 ratio (0.7) phytane/C-18 ratio (0.35) were observed. The relative amounts of any combination of individual aliphatic hydrocarbons can be measured and matched against the crude oil sample from the Gulf of Mexico composition. As illustrated in Figure 4 which shows both direct analysis of crude oil from the Gulf of Mexico as well as analysis after cleanup from oysters. However, it should be noted that the composition of the oil changes with time and the uptake by oysters eventually may have a different profile from the crude oil. The composition of other samples of crude oils is illustrated in Figure 5 again indicating differences in profile.

Similarly the pattern of PAHs found in crude oil is very characteristic as shown in Table 4 with levels of Ant, Phe, Flu and Chr being 100 times higher than levels of B(a)P. Subject to satisfactorily meeting requirements for identification of PAHs, the method gives semi-quantitative values for the higher mass PAHs which can be used as a good guide as to whether oysters samples are above or below safety limits. Accurate results require confirmation using a more refined cleanup procedure.

12. Method Performance

Method performance was established by separate spiking experiments for blank oysters with firstly a mixture of aliphatic hydrocarbon standards (NIST1494 – C10-C34 hydrocarbons) and secondly a mixture of 16 PAH standards. To evaluate method performance with combined aliphatic hydrocarbons and PAHs, spiking was carried out with NIST 1582 petroleum crude oil.

12.1 Recovery

Aliphatic hydrocarbons – The method was shown to be unsuitable for recovery of aliphatic hydrocarbons below n-pentadecane due to losses during concentration of the sample extract. Average recoveries of n-hexadecane (C-16) to n-tetratricontane (C-34) ranged from 52-108%.

PAHs – Background contamination and lack of availability of a real blank sample made it impossible to make an accurate estimate of the recoveries of the lower mass PAHs (Naph, Ace, Acy, Flu, Ant, Phe, Fln and Pyr). However average recoveries of the remaining higher mass PAHs [(B(a)P, Chr, B(b)F, B(k)F, B(k)F, B(a)P, B(g,h,i)P, and D(a,h)A] ranged from 65-126%.

12.2 Specificity

Aliphatic hydrocarbons – Full scan spectra were obtained in each case. Identification was confirmed by close agreement of retention times for standards and comparison with scanned spectra, particularly checking for evidence of interferences. Extracted ion chromatograms using *m/z* 57 were used for profiling but additional ions characteristic of aliphatic hydrocarbons (e.g. *m/z* 71) can be used as an additional check of specificity.

PAHs – By SRM, specificity was confirmed based on the presence of transition ions (quantifier and qualifier) at the correct retention times corresponding to those of the respective PAH standards. Furthermore, the measured peak area ratios of precursor to quantifier ion should be in close agreement with those of the standards.

12.3 Limits of Detection

Aliphatic hydrocarbons – LODs for aliphatic hydrocarbons were estimated to be between 0.2 and 1 ng (on-column injected) in full scan mode. For 1 µL of extract injected into the GC-MS this is equivalent to 20-100 ng/g (ppb) hydrocarbon contamination of the oysters.

PAHs – Background contamination made it impossible to make an accurate estimate of the LODs of the lower mass PAHs (Naph, Ace, Acy, Flu, Ant, Phe, Fln and Pyr). However, LODs of the remaining higher mass PAHs [(B(a)P, Chr, B(b)F, B(k)F, B(k)F, B(a)P, B(g,h,i)P, and D(a,h)A] were estimated to be between 0.01 and 0.07 ng (on-column injected) in SRM mode. For 1 µL of extract injected into the GC-MS/MS this is equivalent to 1-7 ng/g (ppb) PAH and oil contamination of oysters.

12.4 Accuracy

The accuracy for measurement of PAHs was determined by spiking NIST crude oil standard into oysters and following the full extraction and cleanup procedure. Background contamination made it impossible to make an accurate estimate of the recoveries of the lower mass PAHs (Naph, Ace, Acy, Flu, Ant, Phe, Fln and Pyr). However average recoveries of (B(a)A, B(a)P, B(g,h,i)P, and I(1,2,3-c,d)P were 124, 92, 81 and 86 % respectively as shown in Table 3. Bearing in mind that the method is intended as a semi-quantitative screen this accuracy was deemed to be satisfactory.

Segment	Duration (min)	PAH and IS	Retention Time (min)	Precursor Ion	Quantifier Ion	Qualifier Ion	Ion Ratio	Collision Energy
1	10.50	Naph	8.66	127.9	102.0	77.8	0.38	15
2	2.50	Acy	12.13	152.0	151.1	126.0	0.11	10
		Ace	12.35	154.0	153.0	152.0	0.12	10
3	1.50	Fln	13.37	165.9	165.0	162.9	0.05	10
4	3.00	Ant	15.87	178.0	176.0	152.0	0.70	30
		Phe	15.95	178.0	176.0	152.0	0.70	30
		Ant-D10	15.89	188.1	160.2	158.2	0.40	30
5	4.50	Flu	19.13	202.0	201.1	200.1	0.40	10
		Pyr	19.97	202.0	201.0	200.1	0.40	10
6	3.70	B(a)A	23.48	228.1	226.0	202.1	0.15	20
		Chr	23.71	228.1	226.2	202.2	0.15	20
		Chr-D12	23.65	240.2	238.1	215.1	0.11	30
		5MChr	24.98	242.1	241.1	227.5	0.15	30
7	3.80	B(b)F	26.75	252.1	250.1	226.1	0.18	30
		B(k)F	26.82	252.1	250.1	226.1	0.18	30
		B(a)P	27.96	252.1	250.1	226.1	0.18	30
		B(a)P-D12	27.87	264.1	260.1	236.0	0.38	30
8	5.50	I(1,2,3-c,d)P	30.96	276.1	274.0	250.0	0.05	35
		B(g,h,i)P	31.99	276.1	274.0	250.0	0.05	35
		BgP-D12	31.86	288.2	286.1	125.1	0.06	35
		D(a,h)A	30.97	278.0	276.0	226.1	0.05	35

Table 1: Parameters for SRM analysis of PAHs grouped according to Figure 1

Hydrocarbon	Empirical Formula	Molecular Ion	Retention Time
n-decane	$C_{10}H_{22}$	142.1	3.99
n-undecane	$C_{11}H_{24}$	156.2	4.97
n-dodecane	$C_{12}H_{26}$	170.2	6.14
n-tridecane	$C_{13}H_{28}$	184.2	7.30
n-tetradecane	$C_{14}H_{30}$	198.2	8.42
n-pentadecane	$C_{15}H_{32}$	212.2	9.50
n-hexadecane	$C_{16}H_{34}$	226.2	10.51
n-heptadecane	$C_{17}H_{36}$	240.2	11.45
pristane	$C_{19}H_{40}$	268.3	11.24
n-octadecane	$C_{18}H_{38}$	254.3	12.41
phytane	$C_{20}H_{42}$	282.3	12.30
n-nonadecane	$C_{19}H_{40}$	268.3	13.28
n-eicosane	$C_{20}H_{42}$	282.3	14.14
n-docosane	$C_{22}H_{46}$	310.3	15.90
n-tetracosane	$C_{24}H_{50}$	338.3	17.73
n-hexacosane	$C_{26}H_{54}$	366.4	19.56
n-octacosane	$C_{28}H_{58}$	394.4	21.35
n-triacontane	$C_{30}H_{62}$	422.4	23.08
n-dotriacontane	$C_{32}H_{66}$	450.5	24.77
n-tetratricontane	$C_{34}H_{70}$	478.5	26.45

Table 2: Aliphatic hydrocarbons monitored in oysters spiked with NIST 1494

PAH	Assigned Value [ng/g]	Measured Value [ng/g]	Recovery [%]
B(a)A	14.06 ± 1.00	17.39	124
B(a)P	5.52 ± 1.00	5.11	92
I(1,2,3-c,d)P	0.85 ± 0.50	0.69	81
B(g,h,i)P	8.54 ± 0.2	7.37	86

Table 3: Analysis of spiked oysters with NIST 1582 crude oil

Hydrocarbon	Average amount [µg/g] (n=2)	PAH	Average amount [µg/g] (n=2)
n-pentadecane	407	Naph	19
n-hexadecane	1484	Acy	436
n-heptadecane	1329	Ace	96
Pristane	928	Fln	144
n-octadecane	337	Ant	11857
Phytane	118	Phe	11287
n-nonadecane	330	Flu	958
n-eicosane	289	Pyr	547
n-docosane	188	B(a)A	29
n-tetracosane	146	CHR	804
n-hexacosane	82	B(b)F	428
n-octacosane	43	B(k)F	40
n-triacontane	31	B(a)P	2
n-dotriacontane	23	B(g,h,i)P	7
n-tetratricontane	10	I(1,2,3-c,d)P	2
		D(h)A	3

Table 4: Composition of Crude oil from Gulf of Mexico. Characteristic pristane/C-17 ratio (0.7) phytane/C-18 ratio (0.35) were observed.

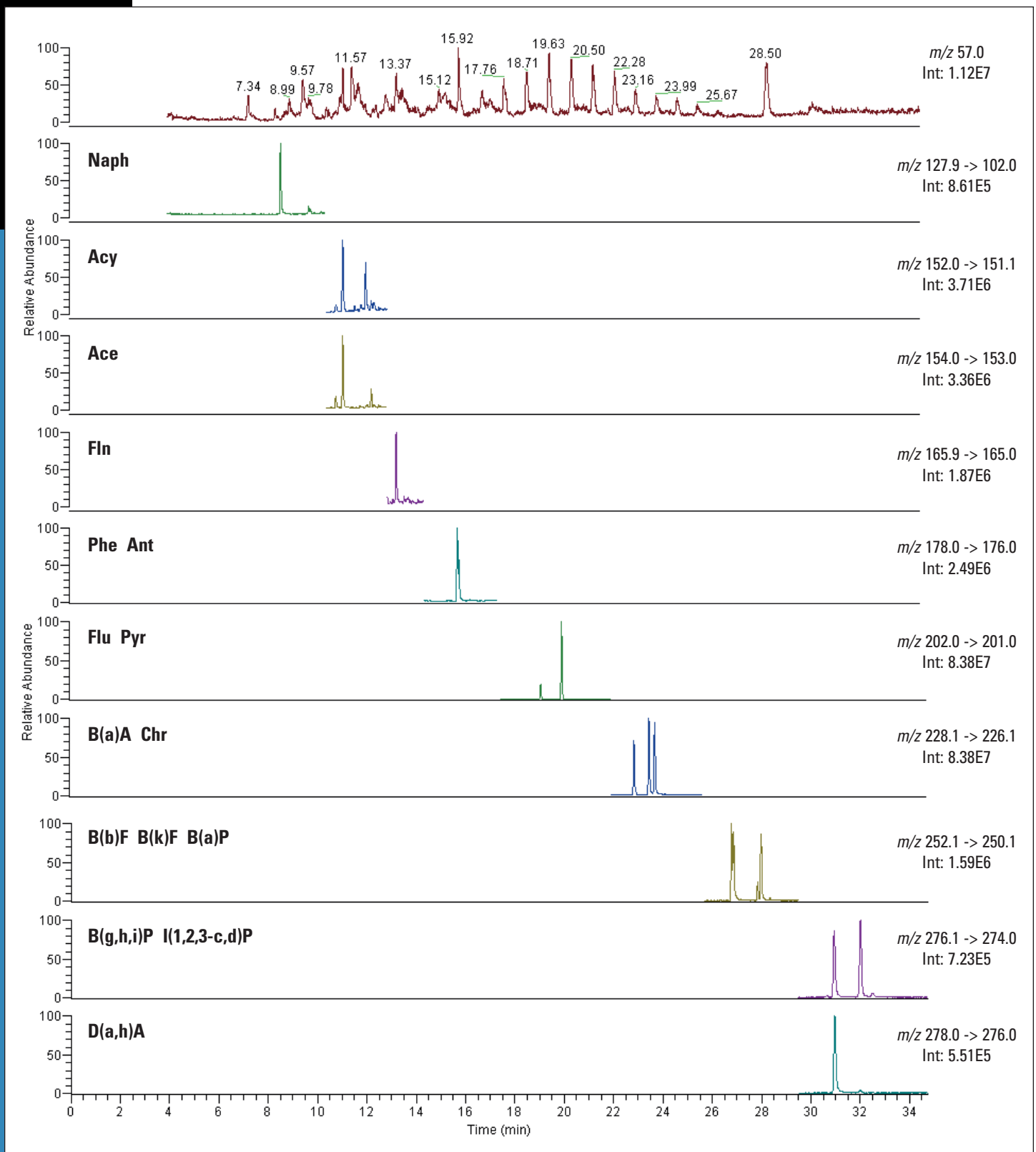


Figure 1: Chromatogram of oyster sample spiked with aliphatic hydrocarbons plus 10 ng/g PAH mixture. Top chromatogram shows *m/z* 57 for hydrocarbon profiling, while lower chromatograms are SRM traces for 16 individual PAHs. Retention times for the 16 PAHs found in Table 1.

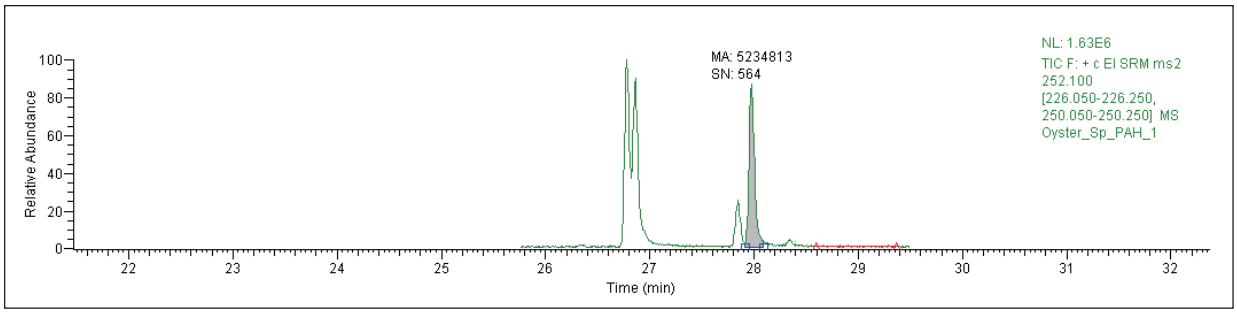


Figure 2: Chromatogram of oyster sample spiked with 10 ng/g B(a)P

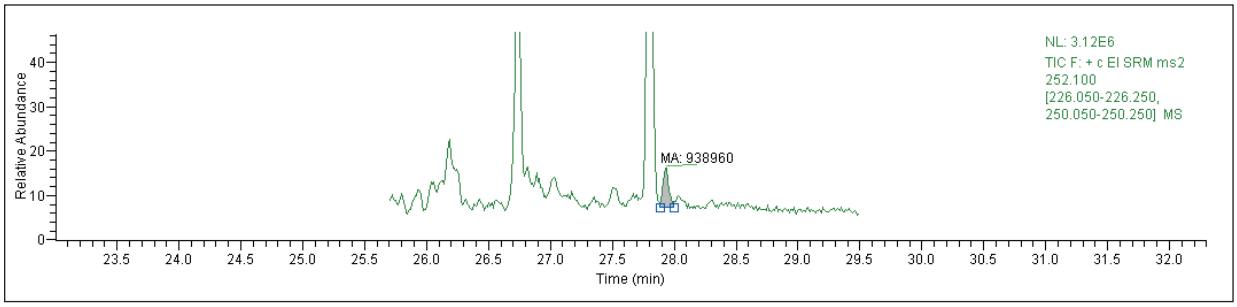


Figure 3: Chromatogram of oyster sample spiked with 5 µg/g crude oil sample taken from the Gulf of Mexico in late May 2010 and found to contain 5 ng/g B(a)P

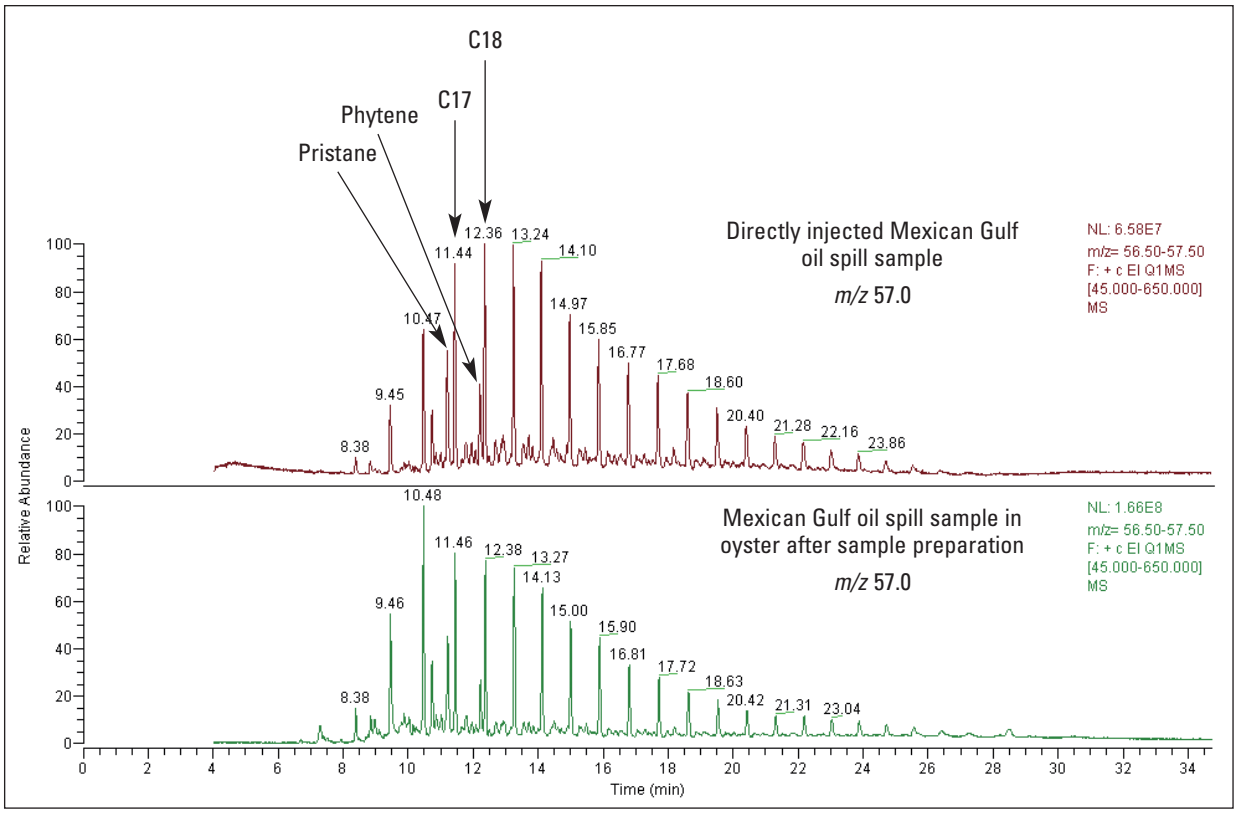


Figure 4: Hydrocarbon profile of crude oil sample taken from the Gulf of Mexico in late May 2010 by direct analysis (top) and after 5 mg/kg spiking into oyster sample (bottom) showing *m/z* 57

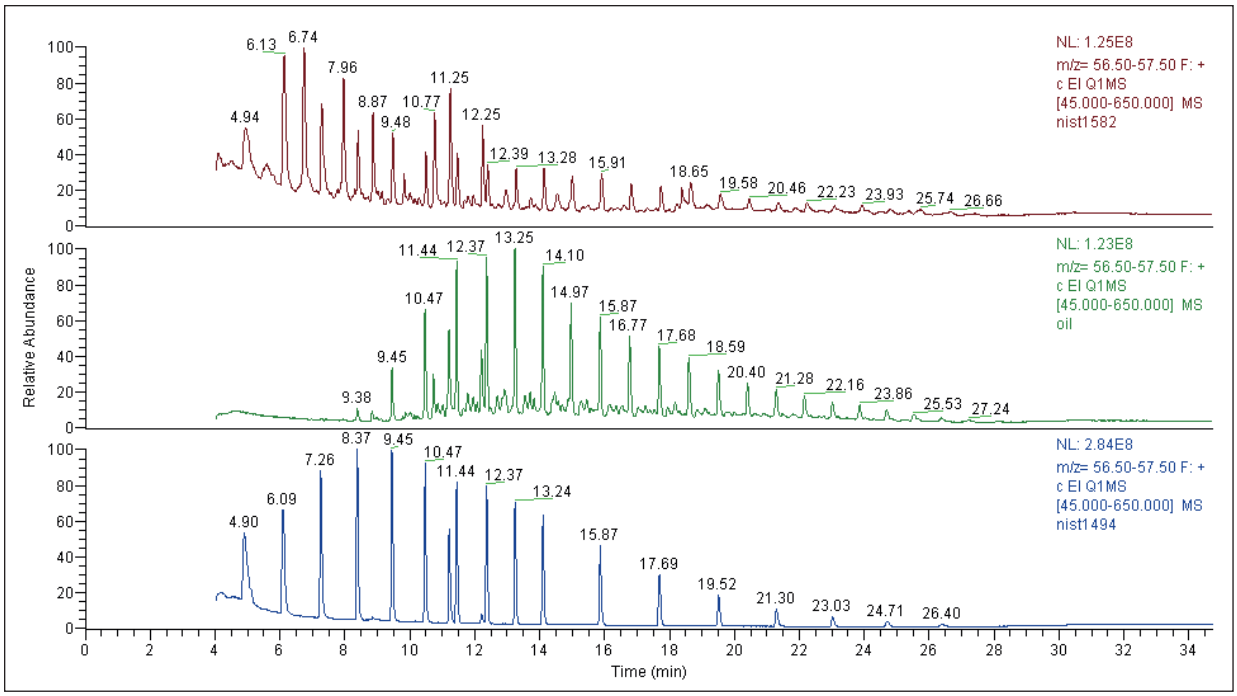


Figure 5: Comparison of hydrocarbon distribution of different type of oils showing *m/z* 57. Top: NIST1582 petroleum crude oil, middle: crude oil sample taken from the Gulf of Mexico in late May 2010, at the bottom: NIST1494 hydrocarbon standard.

TG51980_E 06/10M

Method: 51991

Determination of Polycyclic Aromatic Hydrocarbons (PAHs) and Aliphatic Hydrocarbons in Fish by GC-MS/MS

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Key Words

- TSQ Quantum XLS
- Aliphatic Hydrocarbons
- Fish Extraction
- Gulf Oil Spill
- Oil Contamination
- PAHs

1. Sample Analysis Time

Sample preparation	3 hours
Instrument analysis	35 minutes/sample

2. Schematic of Method

Sample (Fish) Homogenization

Sample 2.0 g + Isotopically Labeled IS

1. Vortex samples (10 s)
2. Equilibrate 15 min

Saponification

3. Add 200 mL MeOH/KOH solution
4. Saponify in u-bath for 30 min/60 °C
5. Cool sample

Extraction and Washing

6. Filter (glass wool) in 500 mL separator funnel
7. Extract with 100 mL hexane
8. Extract a second time MeOH layer with 100 mL of hexane
9. Combine hexane layers in separator funnel
10. Wash twice each time with 50 mL of MeOH/Water mixture

Concentration and Clean-up

11. Evaporate hexane layer to ca. 1 mL
12. Condition SPE with 3 mL hexane
13. Apply sample
14. Elute up to 5 mL with hexane

Concentration

15. Gently evaporate under nitrogen stream to dryness

GC-MS/MS

16. Reconstitute in 180 µL of cyclohexane + 20 µL of Injection/surrogate standard

3. Scope

This method can be applied to fish and other fatty seafood samples to detect simultaneously the presence of aliphatic hydrocarbons and PAH contamination from crude oil found in the Gulf of Mexico. From the profile using GC-MS/MS, the method can be used to characterize the source of contamination. The method gives a quantitative indication as to whether levels of PAHs exceed safety limits for human consumption.

4. Principle

The homogenized fish sample is fortified with appropriate labeled internal standards and saponified with methanolic KOH. After repeated extraction into hexane, further clean-up is carried out on a silica-SPE-cartridge. The concentrated extract is analyzed by GC-MS/MS using a Thermo Scientific TSQ Quantum XLS gas chromatography triple quadrupole mass spectrometer system. PAHs of food safety significance are quantified and compared with the profile from crude oil collected from the Gulf of Mexico in May 2010. Additionally the profile of aliphatic hydrocarbons can be examined.

5. Reagent List

		Fisher Scientific USA Part Number
5.1	Acetone	A9491
5.2	Cyclohexane	C6201
5.3	Hexane	H3021
5.4	Methanol	M/4058/17
5.5	Potassium hydroxide	P/5600/53
5.6	Toluene	AC176850010

Thermo SCIENTIFIC

6. Calibration Standards

6.1 PAHs

Acenaphthene – Ace (Sigma)
Acenaphthylene – Acy (Sigma)
Anthracene – Ant (Sigma)
Benz[a]anthracene – B(a)A (Sigma)
Benzo[a]pyrene - B(a)P (Sigma)
Benzo[b]fluoranthene – B(b)F (Sigma)
Benzo[g,h,i]perylene - B(g,h,i)P (Sigma)
Benzo[k]fluoranthene - B(k)F (Sigma)
Chrysene – Chr (Sigma)
Dibenz[a,h]anthracene - D(a,h)A (Sigma)
Fluoranthene – Flu (Sigma)
Fluorene - Fln (Sigma)
Indeno(1,2,3-cd)pyrene - I(1,2,3-c,d)P (Sigma)
Naphthalene - Naph (Sigma)
Phenanthrene - Phe (Sigma)
Pyrene – Pyr (Sigma)

6.2 Injection Standard

5-methylchrysene – 5-MChr (Dr. Ehrenstorfer)

6.3 Internal Standards

Anthracene-D10 – Ant-D10 (Sigma)
Benzo[a]pyrene-D12 – B(a)P-D12 (Sigma)
Benzo[ghi]perylene-D12 – B(g,h,i)P-D12 (LGC Standards)
Chrysene-D12 – Chr-D12 (Sigma)

6.4 Quality Control Materials

FAPAS, smoked fish (T0642)

Petroleum Crude oil (NIST Standard Reference Material®, 1582)

7. Standards and Reagent Preparation

7.1 MeOH/KOH solution, weigh 120 g KOH, add 60 mL of water and dilute in 900 mL Methanol

7.2 MeOH/H_2O mixture: 400 mL of Methanol plus 100 mL of Water

7.3 Stock solutions of 2 µg/mL of PAH standards in toluene

7.4 Internal PAHs standard (IS) concentration: is 2 µg/mL (Benzo(g,h,i)perylene-d12, Anthracene-d10, Chrysene-d12) in toluene and 200µg/mL Benzo(a)pyrene-d12 in cyclohexane

7.5 Working standard solution mixture of 16 PAHs in toluene (100 ng/mL)

7.6 Working internal standard mixture of IS PAHs in toluene (200 ng/mL)

7.7 Syringe standard, 5-methyl-chyrsene (200 ng/mL) in toluene

7.8 Spike solution of Petroleum crude oil (NIST 1582): 100 mg/mL in cyclohexane

8. Apparatus

		Fisher Scientific USA Part Number
8.1	Centrifuge, Heraeus™ Multifuge™ X3	75-004-500
8.2	Thermo Scientific 16 port SPE vacuum manifold	03-251-252
8.3	Evaporator EVTM-130-32-16 (Fisher Scientific Germany)	3106395
8.4	Fisher precision balance	01918306
8.5	Vacuum pump	05-402-100
8.6	Rotavapor® R-210	05-024-21
8.7	Sartorius analytical balance	01-910-3224
8.8	Thermo Scientific Barnstead EASYpure™ II water	0905050
8.9	Ultrasonic bath Elmsonic S40H	154606Q
8.10	ULTRA-TURRAX® – dispergation tool	1425980
8.11	ULTRA-TURRAX – Plug-in coupling	14259023
8.12	ULTRA-TURRAX	142259301
8.13	Vortex shaker	14505141
8.14	Vortex standard cap	14-505-140
8.15	TSQ Quantum XLS Triple Quadrupole Mass Spectrometer	

9. Consumables

		Part Number
9.1	GC vials	03393F
9.2	Pipette Finnpipette 100-1000 µL	14386320
9.3	Pipette Finnpipette 10-100 µL	14386318
9.4	Pipette Finnpipette 500-5000 µL	14386321
9.5	Pipette holder	14245160
9.6	Pipette Pasteur soda lime glass 150 mm	136786A
9.7	Pipette suction device	03-692-350
9.8	Pipette tips 0.5 – 250 µL, 500/box	21377144
9.9	Pipette tips 1 – 5 mL, 75/box	2137750
9.10	Pipette tips 100 – 1000 µL, 200/box	2137746
9.11	Spatula, 18/10 steel	14356C
9.12	Spatula, nylon	NC9319088
9.13	SPE HyperSep SI, 200 mg/3 mL, 50 pc.	03251270
9.14	Tube holder	03840233
9.15	Wash bottle, PTFE	0340911A
9.16	Glass wool	386062500
9.17	GC column TR-50MS 30m, 0.25 mm ID, 0.25 µm film	260R142P

Glassware *(9. Consumables continued)*

9.18	Beaker, 50 mL	FB100050
9.19	Fisherbrand laboratory bottle, 250 mL	9653630
9.20	Erlenmeyer Flask, 100 mL	9653520
9.21	Fisherbrand test tubes	14-958D
9.22	Funnel, 55mm	14353D
9.23	Glass tubes	14957E
9.24	Measuring cyclinder, 100 mL	FB56449
9.25	Measuring cyclinder, 1000 mL	FB 56453
9.26	Pasteur pipette	136786A
9.27	Round flask 50 mL, NS 29/32 Fisher Scientific Germany	9011835
9.28	Separator funnel, 250 mL	9203325
9.29	Separator funnel, 500 mL	9203328
9.30	Volumetric flask, 10 mL	FB40110
9.31	Volumetric flask, 25 mL	10200A

10. Procedure

10.1 Saponification

10.1.1 Accurately weigh the homogenized sample (ca. 2 g) into a 250 mL Duran Bottle

10.1.2 Add 50 µL of PAH internal standard solution to the sample

10.1.3 Vortex the mixture for 10 s and wait 10 min for equilibration

10.1.4 Add 200 mL of MeOH/KOH solution

10.1.5 Put samples into an ultrasonic bath for 30 min at 60 °C for saponification

10.1.6 Cool sample

10.2 Extraction

10.2.1 Filter saponified sample through glass wool into a 500 mL separating funnel

10.2.2 Add 100 mL of hexane to the sample and shake for 3 min

10.2.3 Transfer the hexane layer into a 100 mL Erlenmeyer flask

10.2.4 Repeat the extraction (10.2.2) one more time

10.2.5 Combine hexane layers in a separator funnel

10.2.6 Wash hexane layer by shaking with 50 mL of MeOH/H_2O solution for 1 min

10.2.7 Repeat washing step (10.2.6) two more times

10.2.8 Evaporate to 1 mL under vacuum (220 mbar/50 °C)

10.3 Clean-up

10.3.1 Condition the SPE-Cartridge with 3 mL of hexane

10.3.2 Apply the extract to the cartridge and elute into an evaporator glass tube with 5 mL of hexane

10.3.3 Evaporate at 40 °C to dryness using a blow-down apparatus under a gentle stream of nitrogen

10.3.4 Reconstitute in 180 µL of cyclohexane plus 20 µL of injection standard

10.4 Analysis

10.4.1 GC operating conditions

GC analysis was performed on a Thermo Scientific TRACE GC Ultra™ system (Thermo Fisher Scientific, Waltham, MA USA). The GC conditions were as follows:

Column: Thermo Scientific TR-50MS 30 m
Column ID: 0.25 mm, 0.25 µm film capillary column
Injection mode: splitless with a 5 mm injection port liner
Injection port temperature: 270 °C
Flow rate: 1.2 mL/min
Split flow: "On", flow: 25 mL/min
Splitless time: 1 min
SSL carrier method mode: constant flow
Initial value: "On" with 1.2 mL/min
Initial time: 1 min
Gas saver flow: 15 mL/min
Gas saver time: 3 min
Vacuum compensation: "On"
Transfer line temperature: 270 °C
Oven Temperature: 60 °C for 1 min, then programmed at 12 °C/min to 210 °C, then 8 °C/min to 340 °C with 5 min hold time

10.4.2 Mass spectrometric conditions

MS analysis is carried out using a Thermo Scientific TSQ Quantum™ XLS triple quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA USA). A satisfactory tune of the mass spectrometer is achieved when the detector is set at *m/z* 300 or less and the three FC 43 (calibration gas) ions (69, 219, and 502) are at least half the height of their respective windows and the ions at 502 and 503 are resolved. The MS conditions were as follows:

Ionization mode: EI positive ion
Ion volume: closed EI
Emission current: 50 uA
Ion source temperature: 250 °C
Scan type: Full scan in range *m/z* 45-650 and SRM
Scan width: 0.15 for SRM
Scan time 0.2 s for full scan and 0.05 for SRM
Peak width: Q1, 0.7 Da; Q3, 0.7 Da FWHM
Collision gas (Ar) pressure: 0.5 mTorr

The mass spectrometer is programmed to be able to simultaneously monitor the hydrocarbon profile in scanning full scan (FS) GC-MS and quantify the presence of PAHs by MS/MS within a single chromatographic run. Eight segments are programmed each with two simultaneous scan events. One scan event is used to monitor the aliphatic hydrocarbon profile throughout the whole chromatographic run (i.e. in all segments), while SRM traces are set up for the target PAHs in the other scan event. The program of segments of SRM events (#1) is shown in Table 1.

Setting of scan event #2 for hydrocarbon profiling was kept constant in all segments:

Scan type: FS in range 45-650 *m/z*
Scan time: 0.2 s
FWHM: 0.7 Da
Collision gas pressure: 0.5

11. Calculation of Results

11.1 Aliphatic Hydrocarbons

Any detectable aliphatic hydrocarbon peaks in Fish can be identified based on their retention times which are given in Table 4. This is illustrated in Figure 4. Measure the specific peak area ratios to characterize the source of hydrocarbon contamination.

11.2 Identification of PAHs

The occurrence of one or more of any of the 16 PAHs of food safety concern is indicated by the presence of transition ions (quantifier and qualifier) as indicated in Table 1 at retention times corresponding to those of the respective standards shown in Table 1. This is illustrated in Figure 1. Careful visual inspection of the SRM chromatograms should be carried out to check for interferences. The measured peak area ratios of precursor to quantifier ion should be in close agreement with those of the standards as shown in Table 1. If the presence of any of the 16 PAHs is confirmed based on retention times and ion ratios then quantification should be carried out as indicated below.

11.3 Quantification of PAHs

Calibration by internal standardization is applied for the quantification of PAHs. This calibration requires the determination of response factors Rf defined by the equation below. Calibration by the internal standardization is applied for the quantification of PAHs. This calibration requires the determination of response factors Rf defined by the equation below.

Calculation of the response factor:

$$R_f = \frac{A_{St} \times c_{[IS]}}{A_{[IS]} \times c_{St}}$$

R_f – response factor determined by the analysis of standards PAH and internal standard

A_{St} – area of the PAH peak in the calibration standard

$A_{[IS]}$ – area of the internal standard peak for the calibration standard

c_{St} – PAH concentration for the calibration standard solution

$c_{[IS]}$ – internal standard concentration for the calibration standard solution

Calculations for each sample the absolute amount of PAH that was extracted from the sample:

$$X_{PAH} = \frac{A_{PAH} \times X_{[IS]}}{A_{[IS]S} \times R_f}$$

X_{PAH} – absolute amount of PAH that was extracted from the sample

A_{PAH} – area of PAH peak of the sample

$A_{[IS]S}$ – area of the internal standard peak of the sample

$X_{[IS]}$ – absolute amount of internal standard added to the sample

The concentration of PAH in the sample (ng/g):

$$c\ (ng/g) = \frac{X_{PAH}}{m}$$

c – concentration of PAH in the sample (ng/g)

m – sample weight in g

12. Interpretation of Results

The analytical data generated in the method requires careful interpretation to collect convincing evidence of aliphatic hydrocarbon contamination of fish originating from actual crude oil sample from Gulf of Mexico and consequent PAH contamination. The method provides a hydrocarbon profile and quantification of PAHs which can be matched against that of crude oil sample from Gulf of Mexico. Although the method provides a PAH profile and simultaneous screening of aliphatic hydrocarbons, it should be noted that the composition of any crude oil contamination may change with time through biodegradation and there may be preferential uptake by fish of individual PAHs and aliphatic hydrocarbon eventually giving a different profile in fish from that of the crude oil.

Subject to satisfactorily meeting the requirements for identification of PAHs the method can be used to quantify levels of PAHs in fish.

13. Method Performance

The method performance was established by spiking experiments with blank oily fish with a mixture of 16 PAH standards The method accuracy was demonstrated first by analysis of surplus proficiency test material samples (FAPAS smoked fish - T0642) with defined PAH values and second by using NIST 1582 petroleum crude oil containing certified levels of PAHs.

13.1 Recovery

Aliphatic hydrocarbons – The method was shown to be unsuitable for recovery of aliphatic hydrocarbons below *n*-hexadecane due to losses during concentration of the sample extract.

PAHs – Average recoveries of the 16 PAHs of food safety significance ranged from 58-113%

13.2 Specificity

Aliphatic hydrocarbons – Full scan spectra were obtained in each case. Identification was confirmed by close agreement of retention times for standards and comparison with scanned spectra, particularly checking for evidence of interferences. Extracted ion chromatograms using *m/z* 57 were used for profiling but additional ions characteristic of aliphatic hydrocarbons (e.g. *m/z* 71) can be used as an additional check of specificity.

PAHs – Using Selected Reaction Monitoring (SRM) the specificity was confirmed based on the presence of transition ions (quantifier and qualifier) at the correct retention times corresponding to those of the respective PAH standards. Furthermore, the measured peak area ratios of qualifier/quantifier ion were in close agreement with the ion ratios of the standards as indicated in Table 1.

13.3 Limits of Detection

The limits of detection in fish were found to be in the range 1-7 ng/g depending on the individual PAH.

13.4 Accuracy

Accuracy was demonstrated by analysis of a smoked fish proficiency test material (FAPAS® T0642) which had assigned values for the significant PAHs. After following the full extraction and cleanup procedure, the FAPAS® sample was analyzed by GC-MS/MS and the results are shown in Table 2. Average recoveries of (B(a)A, B(a)F, B(a)P, I(1,2,3-c,d)P and B(g,h,i)P were 101, 96, 97, 116 and 94 % respectively. The accuracy of this method for these critical PAHs in fish was thus demonstrated.

Accuracy was demonstrated by analysis of blank fish spiked with a solution of NIST crude oil containing certified levels of PAHs. After following the full extraction and cleanup procedure, samples were analyzed by GC-MS/MS and the results are shown in Table 3. Average recoveries of (B(a)A,, B(a)P, I(1,2,3-c,d)P and B(g,h,i)P were 107, 112, 82 and 112 % respectively. The accuracy of this method for determining these critical PAHs in fish in the presence of crude oil was thus demonstrated.

Segment	Duration (min)	PAH and IS	Retention Time (min)	Precursor Ion	Quantifier Ion (*m/z*)	Qualifier Ion (*m/z*)	Ion Ratio	Collision Energy (V)
1	10.50	Naph	8.65	127.9	102.0	77.8	0.38	15
2	2.50	Acy	12.12	152.0	151.1	126.0	0.11	10
		Ace	12.34	154.0	153.0	152.0	0.12	10
3	1.50	Fln	13.36	165.9	165.0	162.9	0.03	10
4	3.00	Ant	15.84	178.0	176.0	152.0	0.70	30
		Ant-D10	15.86	188.1	160.2	158.2	0.38	30
		Phe	15.91	178.0	176.0	152.0	0.65	30
5	4.50	Flu	19.08	202.0	201.1	200.1	0.18	10
		Pyr	19.93	202.0	201.0	200.1	0.17	10
6	3.70	B(a)A	23.47	228.1	226.0	202.1	0.15	20
		Chr-D12	23.62	240.2	238.1	215.1	0.11	30
		Chr	23.70	228.1	226.2	202.2	0.15	20
		5MChr	24.94	242.1	241.1	227.5	0.15	30
7	3.80	B(b)F	26.72	252.1	250.1	226.1	0.18	30
		B(k)F	26.80	252.1	250.1	226.1	0.18	30
		B(a)P-D12	27.85	264.1	260.1	236.0	0.38	30
		B(a)P	27.93	252.1	250.1	226.1	0.18	30
8	5.50	I(1,2,3-c,d)P	30.91	276.1	274.0	250.0	0.05	35
		D(a,h)A	30.93	278.0	276.0	226.1	0.05	35
		B(g,h,i)P-D12	31.84	288.2	286.1	125.1	0.06	35
		B(g,h.i)P	31.95	276.1	274.0	250.0	0.05	35

Table 1: Parameters for SRM analysis of PAHs grouped according to Figure 1

PAH	Assigned Value [ng/g]	Satisfactory Range	Measured Value [ng/g]	Recovery [%]
B(a)A	6.35	3.56 - 9.14	6.43	101
B (b)F	1.31	0.73 - 1.89	1.26	96
B (a)P	3.41	1.91 - 4.90	3.32	97
I (1,2,3-cd)P	2.53	1.42 - 3.64	2.38	94
B (g,h,i)P	4.37	2.45 - 6.30	5.11	116

Table 2: Analysis of FAPAS® smoked fish T0642 proficiency test material (values given in ng/g, n=4)

PAH	Assigned Value [ng/g]	Measured Value [ng/g]	Recovery [%]
B(a)A	28.12	30.20	107
B(a)P	11.05	12.40	112
I(1,2,3-c,d)P	1.71	1.40	82
B(g,h,i)P	17.07	17.40	102

Table 3: Analysis of spiked fish with NIST 1582 crude oil (values given in ng/g, n=4)

Hydrocarbons	Emprical Formula	Molecular Ion	Retention Time
n-hexadecane	$C_{16}H_{34}$	226.2	10.48
n-heptadecane	$C_{17}H_{36}$	240.2	11.47
pristane	$C_{19}H_{40}$	268.3	11.26
n-octadecane	$C_{18}H_{38}$	254.3	12.39
phytane	$C_{20}H_{42}$	282.3	12.29
n-nonadecane	$C_{19}H_{40}$	268.3	13.29
n-eicosane	$C_{20}H_{42}$	282.3	14.15
n-heneicsosane	$C_{21}H_{44}$	296.3	15.03
n-docosane	$C_{22}H_{46}$	310.3	16.00
n-tricosane	$C_{23}H_{48}$	324.3	16.83
n-tetracosane	$C_{24}H_{50}$	338.3	17.73
n-pentacosane	$C_{25}H_{52}$	352.4	18.80
n-hexacosane	$C_{26}H_{54}$	364.4	19.54
n-heptacosane	$C_{27}H_{56}$	378.4	20.45
n-octacosane	$C_{28}H_{58}$	394.4	21.30
n-nonacosane	$C_{29}H_{60}$	408.4	22.20
n-triacontane	$C_{30}H_{62}$	432.4	23.02

Table 4: Aliphatic hydrocarbons monitored in fish spiked with crude oil from Gulf of Mexico

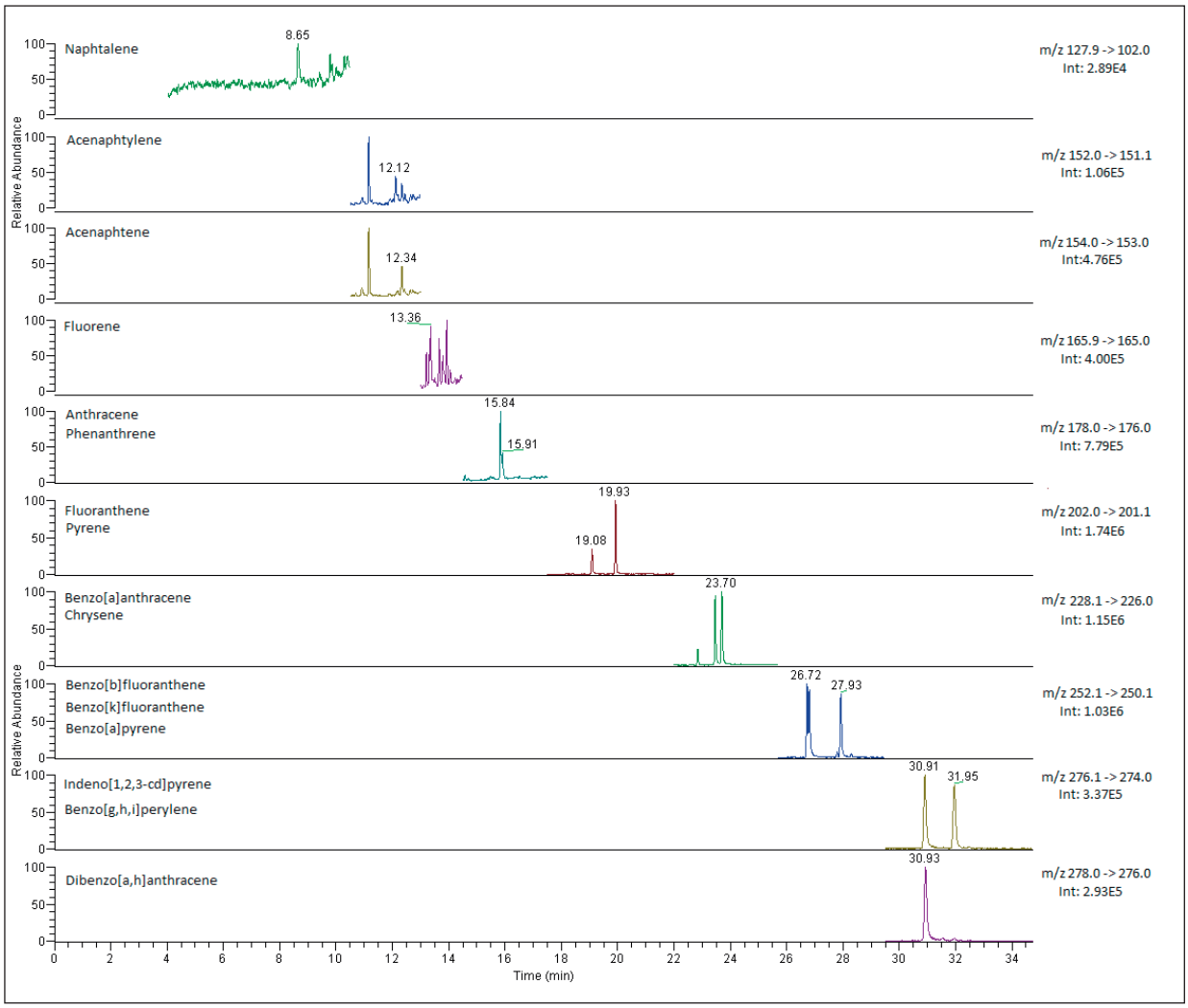

Figure 1: Chromatogram of red snapper fish sample spiked with 5 ng/g PAHs

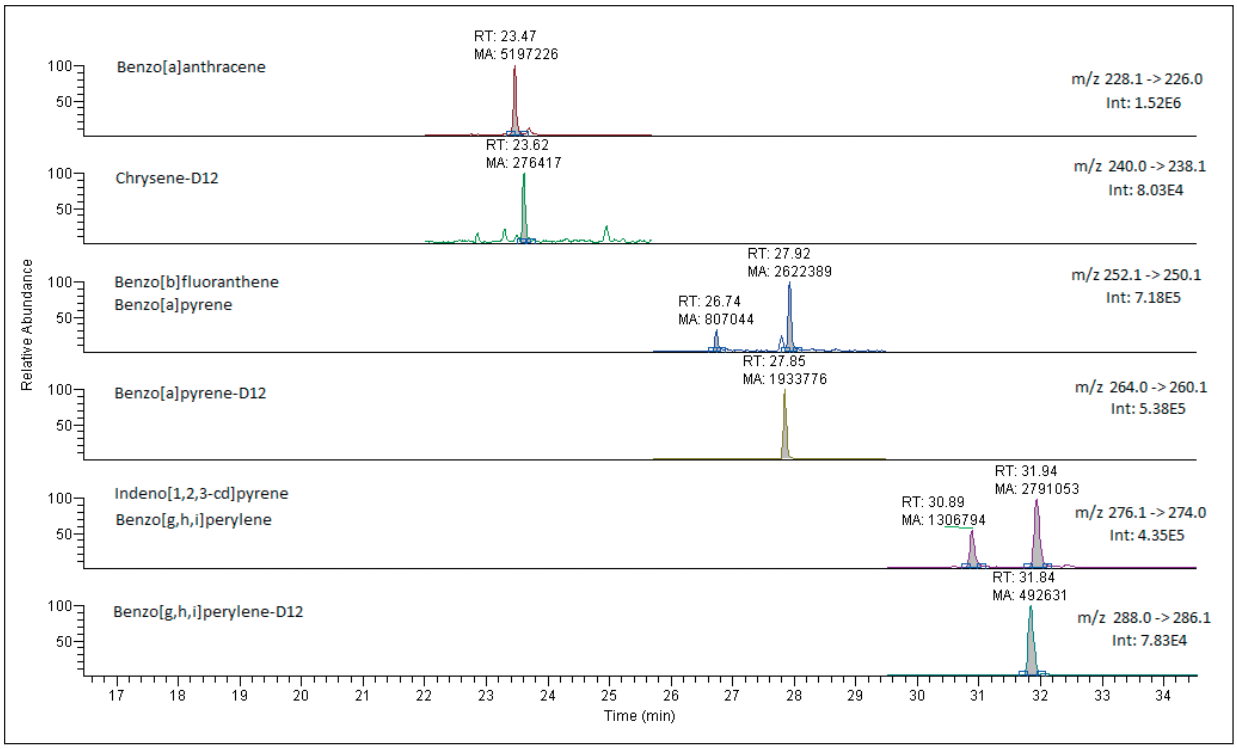

Figure 2: Chromatogram of FAPAS® T0642 smoked fish quality control sample showing peaks of the measured B(a)A, B(b)F, B(a)P, B(g,h,i)P, I(1,2,3-c,d)P PAHs and the respective internal standards

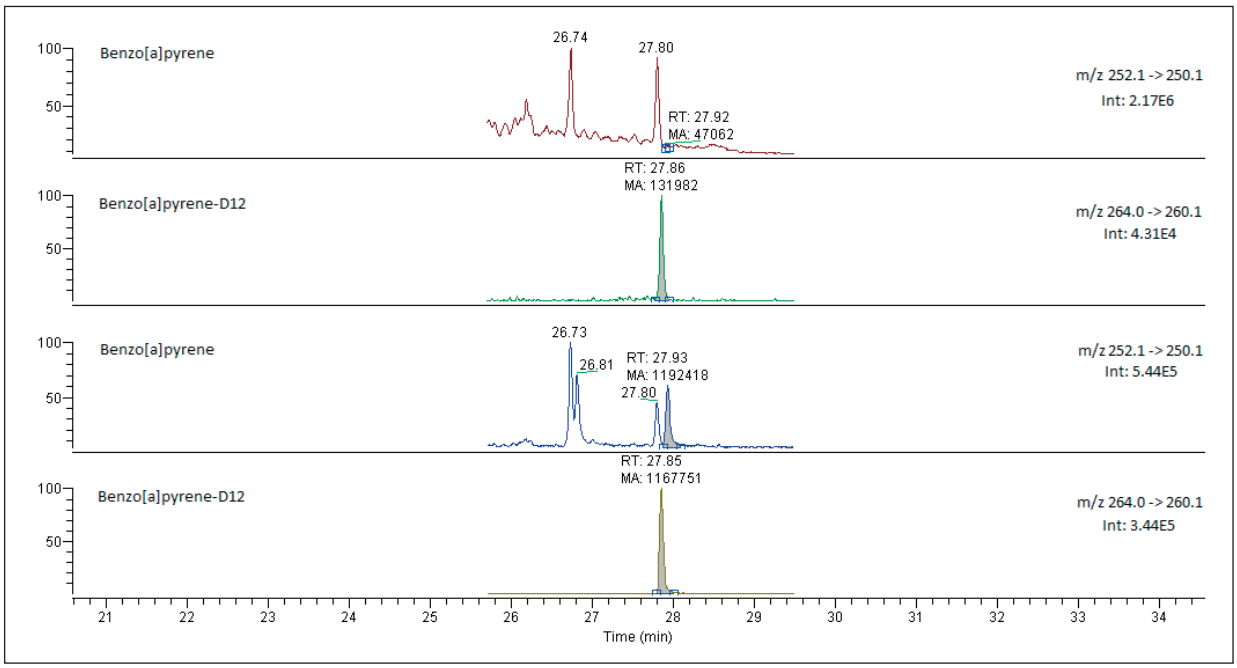

Figure 3: Chromatogram of *m/z* 252.1 -> 250.1 transition (for B(b)F, B(k)F and B(a)P- marked) in red snapper fish sample spiked with the actual oil spill sample from the Gulf of Mexico. Last two chromatograms representing the same sample (same transition and the relevant internal standard) spiked with standard addition at 5 ng/g PAHs concentration level.

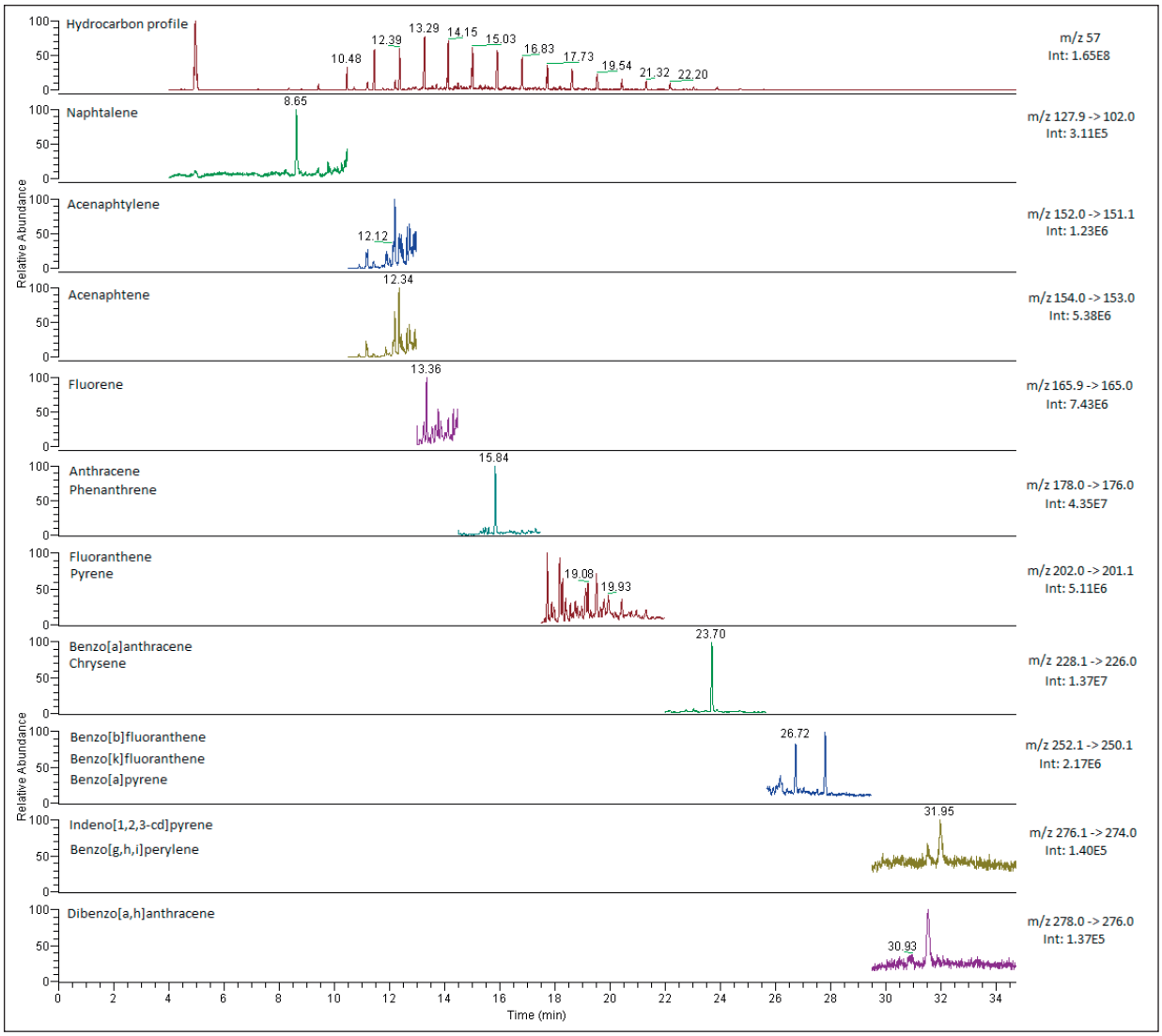

Figure 4: Chromatogram of red snapper fish sample spiked with actual oil spill sample (5 mg/g fish) from the Gulf of Mexico. Retention times indicate PAHs found in the sample.

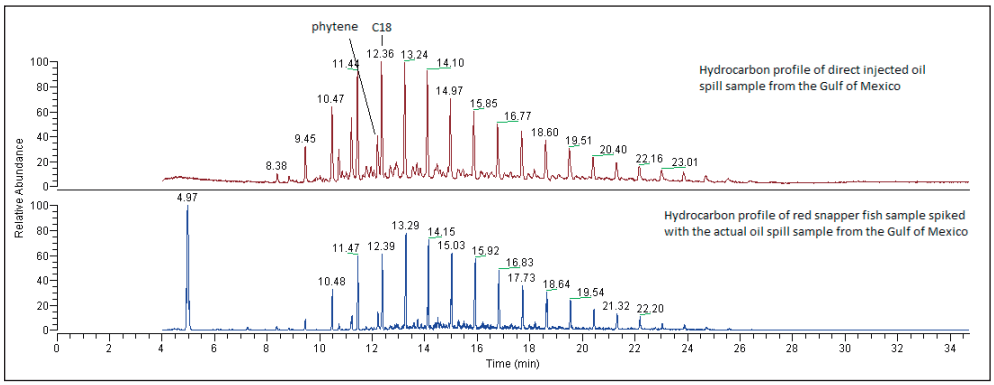

Figure 5: Hydrocarbon profile at *m/z* 57 of actual oil spill sample taken from the Gulf of Mexico after direct injection (top) and after spiking it at 5 mg/g fish concentration level into red snapper fish sample (bottom).

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Application Note: 52266

High Efficiency, Quantitative Dioxins Screening at the Level of Interest in Feed and Food using Advanced GC-MS/MS

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Key Words

- Compliance
- Confirmation
- Dioxins
- GC-MS/MS
- PCBs
- Screening

Introduction

Removing the frequency of contamination events caused by dioxins and dioxin like substances is a high priority for governments and organizations charged with the task of protecting human health. The largest source of human dioxin exposure comes though dietary intake of food of animal origin. Consequently, there are extensive monitoring programs in place to identify potential contamination entering into the food chain.[1]

When contamination is discovered at non-compliant levels (above maximum levels allowed) the consequences can be serious and widespread. Apart from the risk to human health, contamination events can have a huge economic and political impact and receive a very high level of media attention. As this is the case, there is a strong need for organizations that interact with the food chain, from food ingredient and feed manufacturers, through to consumer suppliers and regulatory bodies to more closely monitor their own interest. The result is that the testing requirement is growing, as is the burden on confirmatory analysis capacity using high resolution (GC-HRMS) techniques.

Current European Union regulations permit the use of GC-MS/MS and bioassay techniques for screening dioxins and dioxin-like PCBs at the level of interest in feed and food samples.[2] GC coupled with triple quadrupole MS is particularly suitable screening technique as isotope dilution is retained as well as the high selectivity of the MS/MS experiment. If results are determined to be at a significant level (non compliant) then confirmatory analysis by a high resolution technique that meets the regulatory requirement must be carried out. In order for a screening technique to be suitable for regulatory dioxins analysis, it must comply with the specific regulations for screening methods and carry with it the ability to strongly correlate with the current "gold standard" confirmatory technique in analytical performance and quality control. These minimum requirements for Total-TEQ (toxic equivalent quotient) from the aforementioned regulations are given in Table 1.

	Screening Methods	Confirmatory Methods
False Negative Rate	<1%	–
Trueness	–	-20% to +20%
Precision (RSDR)	<30%	<15%

Table 1: Commission Regulation (EC) No 152/2009 (Feed), No 1883/2006 (Food)

This application note describes the use of the Thermo Scientific TSQ Quantum XLS Ultra GC-MS/MS as applied to high efficiency screening of PCDDs/PCDFs in feed and food samples at the levels of interest and the level of agreement with "gold standard" confirmatory analysis using GC-HRMS (Thermo Scientific DFS).

Materials and Methods

Extraction and Clean-up

The extraction and clean-up process for food and feed samples was performed according Figure 1. For food samples with legal limits on fat basis, the application of a maximum of 3 g of fat for clean-up is applied for achieving low limits of quantification with this method.

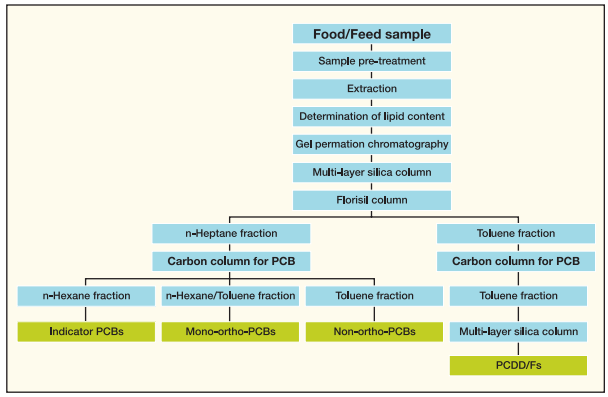

Figure 1: Extraction and clean-up for determination of PCDD/Fs and PCBs in food and feed samples

GC-MS measurement

The GC-MS/MS measurements were performed using a TSQ Quantum XLS Ultra™ GC-MS/MS system.

The following MS/MS settings were applied:

Source Temperature	250 °C
Ionization	EI
Electron Energy	40 eV
Emission Current	50 µA
Q2 Gas Pressure (Argon)	1.5 mTorr
Collision Energy	22 V
Q1 Peak Width	0.7 amu
Q3 Peak Width	0.7 amu

Table 2: Mass spectrometer parameters

The collision cell (Q2) gas pressure and collision energy were optimized for PCDD/F measurement. The monitored SRM transitions as well as the GC conditions are given below in Table 3.

PCDD/F	Precursor	Product
TCDF	303.90	240.94
TCDF	305.90	242.94
^{13}C TCDF ISTD	315.94	251.97
^{13}C TCDF ISTD	317.94	253.97
TCDD	319.90	256.90
TCDD	321.89	258.89
^{13}C TCDD ISTD	331.94	267.97
^{13}C TCDD ISTD	333.93	269.97
PeCDF	339.86	276.90
PeCDF	341.86	278.89
^{13}C PeCDF ISTD	351.90	287.93
^{13}C PeCDF ISTD	353.90	289.93
PeCDD	355.85	292.85
PeCDD	357.85	294.85
^{13}C PeCDD ISTD	367.90	303.90
^{13}C PeCDD ISTD	369.89	305.89
HxCDF	371.82	308.86
HxCDF	373.82	310.86
^{13}C HxCDF ISTD	383.86	319.90
^{13}C HxCDF ISTD	385.86	321.89
HxCDD	387.82	324.82
HxCDD	389.82	326.82
^{13}C HxCDD ISTD	399.86	335.86
^{13}C HxCDD ISTD	401.86	337.86
HpCDF	407.78	344.82
HpCDF	409.78	346.82
^{13}C HpCDF ISTD	419.82	355.86
^{13}C HpCDF ISTD	421.82	357.85
HpCDD	423.78	360.78
HpCDD	425.77	362.77
^{13}C HpCDD ISTD	435.82	371.82
^{13}C HpCDD ISTD	437.81	373.81
OCDF	441.76	378.80
OCDF	443.76	380.79
^{13}C OCDF ISTD	453.78	389.82
^{13}C OCDF ISTD	455.78	391.81
OCDD	457.74	394.74
OCDD	459.74	396.74
^{13}C OCDD ISTD	469.78	405.78
^{13}C OCDD ISTD	471.78	407.78

Table 3: Target congener groups SRM transitions

The results of the GC-MS/MS measurements were compared with routine GC-HRMS measurements using the DFS High Resolution MS (Thermo Scientific, Bremen, Germany).

PTV Injection (PCDD/Fs)	
Injected Volume	5 µL (toluene)
Injection Speed	5 µL/s
Liner	Open Silcosteel® liner (Restek®)
Injection Temperature	100 °C
Vent Flow	20 mL/min
Transfer Rate	13.3 °C/s
Final Transfer Temperature	340 °C
GC Programme (PCDD/Fs)	
GC Column	DB-5MS (60 m, 0.25 µm, 0.25 mm)
Initial Temperature	120 °C
Rate 1	17 °C/min to 250 °C
Rate 2	2.5 °C/min to 285 °C
Final Temperature	285 °C for 13 min

Table 4: GC and injector conditions

Results and Discussion

Selectivity, Sensitivity and Quantitative Performance

In order for a screening technique to be truly efficient it needs to be able to perform at a level that closely correlates with high resolution confirmatory techniques. The first prerequisite of any such technique is sensitivity and selectivity. Figure 2 shows an overlay of 2,3,7,8-TCDD target ions for five injections of a mixed animal fat sample at 0.13 pg/g fat. The sensitivity and selectivity obtained was high enough to allow comfortable, precise detection with all ion ratio integrity maintained. Figure 3 shows overlay of 1,2,3,7,8-PeCDF (0.4 ng/kg 88% dry weight) and 2,3,4,7,8-PeCDF (3.4 ng/kg 88% dry weight) for four injections of grass meal (animal feed) sample. Figure 4 shown native PCDD/Fs SRM chromatograms for the bottom calibration level for this methodology.

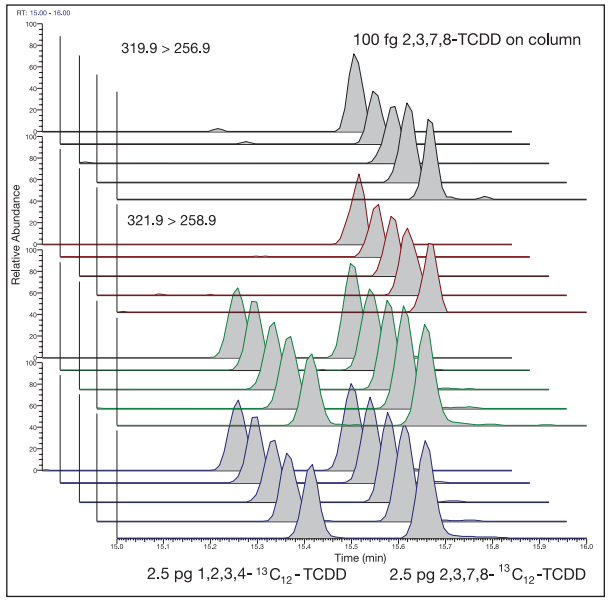

Figure 2: Overlay of 2,3,7,8-TCDD target ions for five injections of a mixed animal fat sample at 0.13 pg 2,3,7,8-TCDD/g fat. 12% CV was achieved on the real calculated amount.

Ion Ratio Confirmation

Most frequently, during routine dioxins analysis using HRMS, an ion ratio comparison of a detected congener is performed against theoretically calculated values. If the value obtained is within acceptable tolerance then the peak has passed that part of the confirmation check. In GC-MS/MS analysis, because of the nature of having two stages of MS, the ion ratios differ from that of HRMS but still form a predictable pattern in line with the isotopic composition of precursor and product masses. This allows high confidence in a strong pre-confirmation positive detection. Figure 4 shows the theoretically calculated ion ratios for SRM analysis of tetra thru octa PCDD/F congeners as well as the measured values obtained from a calibration sequence using the TSQ Quantum XLS Ultra. The data obtained showed strong agreement, well within a typical ±15% QC tolerance (comparable to QC tolerances for GC/HRMS methods in EPA Method 1613 revision B).

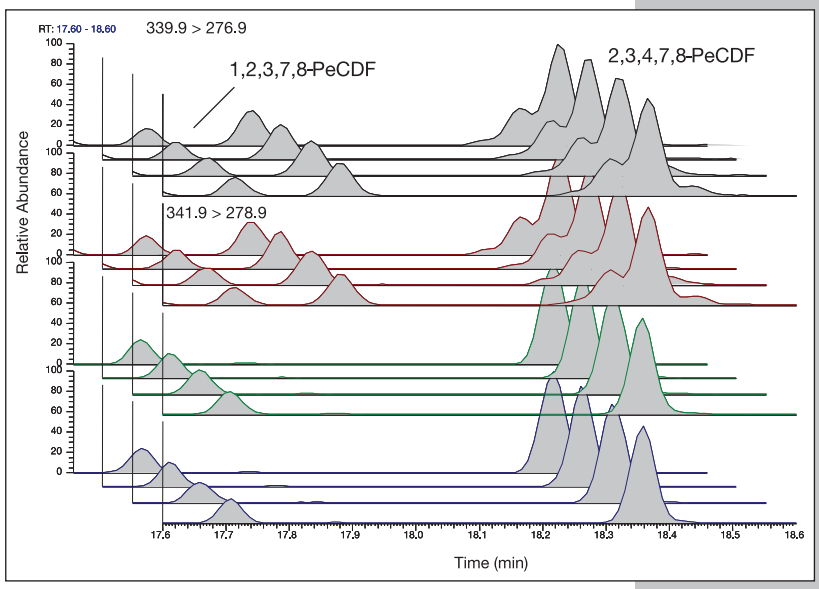

Figure 3: Overlay of 1,2,3,7,8-PeCDF (0.4 ng/kg 88% dry weight) and 2,3,4,7,8-PeCDF (3.4 ng/kg 88% dry weight) for four injections of grass meal sample

Sample and QC Information

Another advantage of screening dioxins using GC-MS/MS is that the isotope dilution quantification technique, common in HRMS confirmatory analysis is retained. This means that solid quantitative data can be achieved, with real TEQ calculations, as well as a good understanding of sample preparation efficiency through recovery information. Table 5 gives recovery information for a set of food samples screened using TSQ Quantum XLS Ultra. In addition, congener provenance with profile information remains with triple quadrupole screening, which can add value to continuous monitoring data. This information is lost in non-GC/MS based screening techniques.

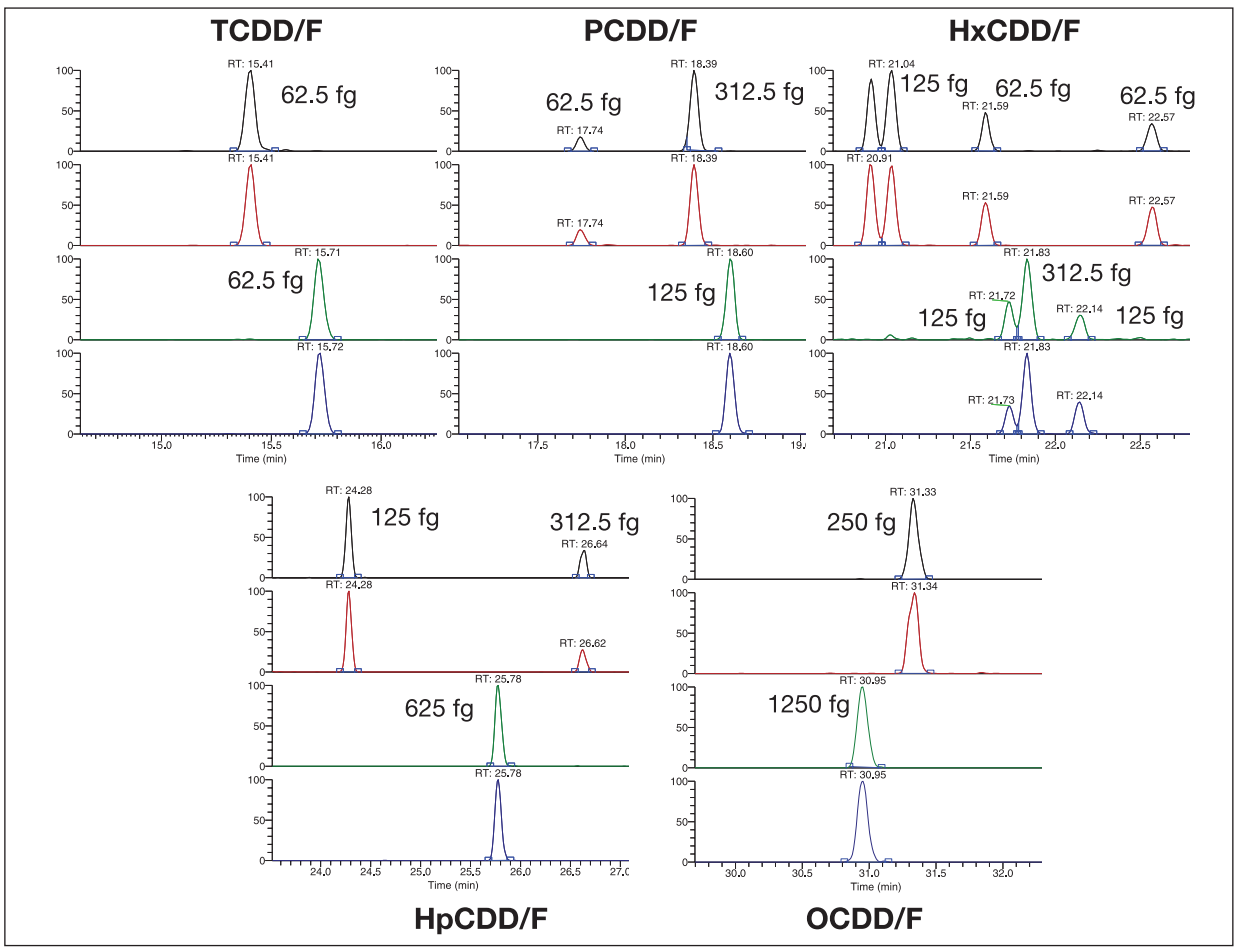

Figure 4: SRM chromatograms of native PCDD/F congeners from the lowest calibration level for the analysis. On column injected amounts are given for each congener. Dibenzofurans can been observed in the top two traces for each congener group and dibenzodioxins in the bottom two.

	Mean Recovery (%)	Relative Standard Deviation (%)
2,3,7,8-TCDF	83	11
1,2,3,7,8-PeCDF	105	12
2,3,4,7,8-PeCDF	101	13
1,2,3,4,7,8-HxCDF	106	14
1,2,3,6,7,8-HxCDF	107	15
2,3,4,6,7,8-HxCDF	104	17
1,2,3,7,8,9-HxCDF	97	17
1,2,3,4,6,7,8-HpCDF	105	18
1,2,3,4,7,8,9-HpCDF	99	18
OCDF	90	26
2,3,7,8-TCDD	87	12
1,2,3,7,8-PeCDD	105	13
1,2,3,4,7,8-HxCDD	110	13
1,2,3,6,7,8-HxCDD	108	14
1,2,3,7,8,9-HxCDD	104	16
1,2,3,4,6,7,8-HpCDD	104	17
OCDD	94	24

Table 5: Recoveries of ^{13}C-labeled internal PCDD/F standards for food samples (n = 42)

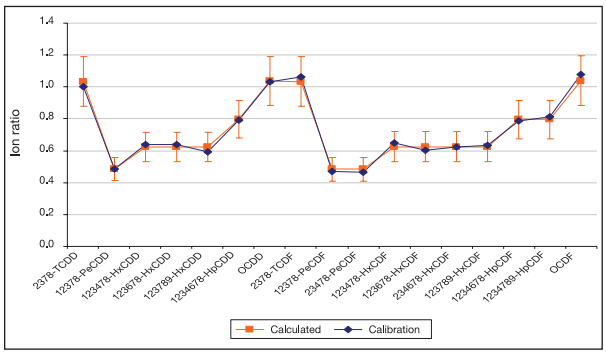

Figure 5: Theoretically calculated ion ratios for SRM analysis of tetra thru octa PCDD/F congeners in addition to the real values obtained from a calibration sequence using the TSQ Quantum XLS Ultra. Error bars show typical ±15% QC tolerance.

Screening Efficiency

A direct comparison of calculated WHO-PCDD/F-TEQ in pg/g fat (or wet weight for fish) was made by analyzing the same sample extracts on both the TSQ Quantum XLS Ultra and the DFS HRMS. The data obtained are given in Figure 6. Very good correlation with HRMS data was observed in the real calculated values down to ca. 0.5 (WHO-PCDD/F-TEQ) pg/g level indicating that a highly

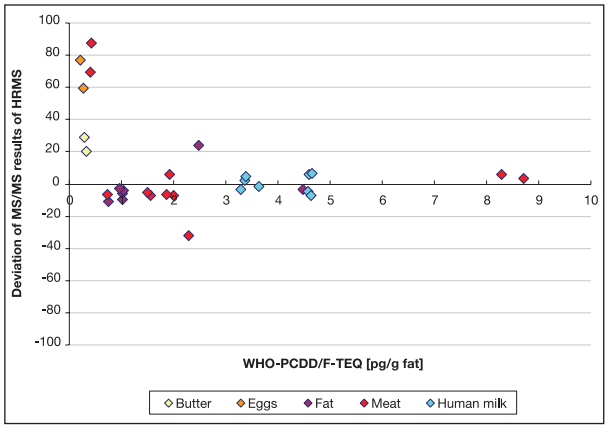

Figure 6: Deviations of WHO-PCDD/F-TEQ of GC-MS/MS results of GC-HRMS (%) for food and human milk samples

efficient screening method is possible with TSQ Quantum XLS Ultra. The sensitivity and selectivity obtained with the technique made this possible. This means, in addition to a very low false negative rate, very few compliant samples are likely to be directed to subsequent confirmatory analysis.

Conclusions

- The Thermo Scientific TSQ Quantum XLS Ultra is a highly applicable screening tool for PCDD/Fs in food and feed.
- Strong correlation, between the results of GC-MS/MS and GC-HRMS within acceptable limits were observed around the level of interest for a high percentage of the food and feed samples tested.
- Measured ion ratios for identity confirmation are predictable and can therefore be tested against theoretical values.
- A different approach for LOQ calculation (from the signal/noise ratio, employed on HRMS systems) is required due to the inherent low noise of the GC-MS/MS system. For this, the lowest calibrated concentration was used.

References

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Application Notebook

Natural Compounds and Food Additives

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GC-MS/MS Analysis of the Receptor-Sensitizing Natural Active Spice Ingredients Capsaicin, Piperine, and Thymol

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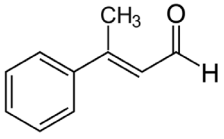
[1]Alpha Analytical Pte. Ltd., Singapore, [2]Thermo Fisher Scientific, Singapore

Keywords: Spices, active ingredients, traditional Chinese medicine TCM, medical applications, personal defense products, pepper spray, MRM, analyte protectants.

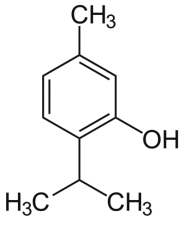
Introduction

Pungent spices are common ingredients for food preparations in all cooking traditions. Spices have been used as well for a long time in the traditional Chinese medicine (TCM). Beyond that there is a modern use of the active ingredients of spices in a variety of personal defense and law enforcement products, such as pepper spray, due to their immediate physiological irritation effects.

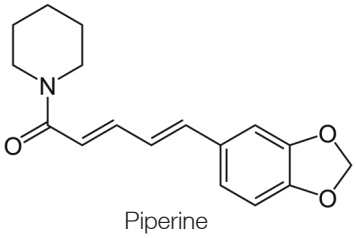
Many of these active ingredients interact with specific receptors and modulate the sensing mechanism of the human body [1]. Such receptors can respond to chemical stimuli caused by a variety of natural and synthetic compounds. Upon receptor activation the nerve signal is interpreted as a painful burning, a sensation consumers of hot dishes recognize [2]. Receptor sensitizing and activation



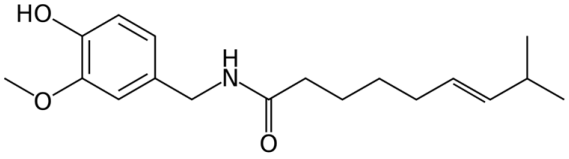
α -Methyl-t-Cinnamaldehyde



Thymol



Piperine



Capsaicin

can be caused by a number of compounds of natural origin that, along with many others, include capsaicinoids, piperine, and thymol.

Capsaicin (CAS 404-86-4) and related capsaicinoid compounds like dihydrocapsaicin (CAS 19408-84-5) occur in plants from the genus Capsicum and are typical of hot (chili) and non-pungent (bell) peppers. Due to its stimulating characteristics, capsaicin is a banned substance in equestrian sports. Piperine (CAS 94-62-2) belongs to a group of alkaloids typical of plants from the Piperaceae family, like black pepper (Piper nigrum L.), a most popular spice. Thymol (CAS 89-83-8) is a naturally occurring monoterpene phenol from Thymus vulgaris, known for its distinctive, strong flavor. Due to its antimicrobial attributes it is also used as an antiseptic ingredient in household products. Trans-cinnamaldehyde (CAS 104-55-2) occurs naturally in the bark of cinnamon trees and other species of the genus Cinnamomum and gives the cinnamon powder its typical flavor and odor. The best known application for cinnamaldehyde is flavoring, but it is also used as a fungicide and an antimicrobial [3]. It is included here as it is known to be used in pepper spray products as a flavoring component.

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This application note describes the GC-MS/MS analysis of extracts from spices as a highly selective tool for the quantitative determination of the representative ingredients of natural active spice ingredients capsaicin, piperine, thymol, and cinnamaldehyde.

Experimental Conditions

All measurements have been carried out using the Thermo Scientific™ TSQ 8000™ triple quadrupole GC-MS/MS system equipped with the Thermo Scientific™ TRACE™ 1310 GC with SSL Instant Connect™ SSL module and Thermo Scientific™ TriPlus™ RSH autosampler. The method details are given in Table 1.

The TSQ 8000 MS acquisition method has been developed automatically by AutoSRM, a unique MS/MS method development tool included in the TSQ 8000 software suite. The AutoSRM method development starts from a standard solution vial in the TriPlus RSH autosampler and automatically determines retention time, the two most suitable precursor and product ions, and optimizes the collision energy for best sensitivity. The program runs automatically and provides the SRM acquisition method based on the timed-SRM mode. The choice of timed-SRM dispenses with the tedious manual search and setting of several segment breaks, as required by former triple quadrupole systems. Timed-SRM uses a short window around the compound retention time, the duration of which is user definable. Once the AutoSRM process is completed, the generated acquisition method as shown in Table 2 is used immediately for sample analysis.

Figure 1. TSQ 8000 with TRACE 1310 GC and TriPlus RSH autosampler

The molecular structures of the natural active compounds under investigation in this application note have very polar groups. These polar sites pose a special challenge to the GC system because of their active nature and long-term instability, especially in real life matrix samples.

Table 1. TRACE 1310 GC and TSQ 8000 MS/MS method parameter

TRACE 1310 GC	
Injection mode	splitless
Splitless Time	1.0 min
GC Column	Restek™ RTX™-5Sil MS, 15 m × 0.25 mm × 0.25 µm
Carrier gas	He (99.999 %)
Flow	1.2 mL/min, constant flow
Temperature program	50 °C, 2 min 20 °C/min to 300 °C, 2 min
Transfer line temperature	280 °C
Total analysis time	14.6 min
TriPlus RSH Autosampler	
Injection volume	1 µL
TSQ 8000 MS/MS	
Ionization mode	EI, 70 eV
Ion source temperature	250 °C
Scan mode	SRM using timed SRM
SRM transition setup	automatically build-up by AutoSRM software, transitions see Tab.2

Table 2. MRM acquisition method created by AutoSRM

Compound name	CAS Number	RT	Precursor Mass	Product Mass	Collision Energy	Peak Width
		[min]	[m/z]	[m/z]	[V]	[min]
Thymol	89-83-8	6.24	135.1	91.1	15	5
Thymol	89-83-8	6.24	150.1	135.1	10	5
α-Methyl-trans-cinnamadehyde	101-39-3	6.51	145.1	91.1	25	5
α -Methyl-trans-cinnamadehyde	101-39-3	6.51	145.1	115.1	20	5
Capsaicin	404-86-4	12.64	137.0	94.0	20	5
Capsaicin	404-86-4	12.64	137.0	122.0	15	5
Dihydrocapsacin	19408-84-5	12.89	137.0	94.0	20	5
Dihydrocapsacin	19408-84-5	12.89	137.0	122.0	15	5
Piperine	94-62-2	14.08	200.8	115.1	20	5
Piperine	94-62-2	14.08	285.0	172.7	10	5

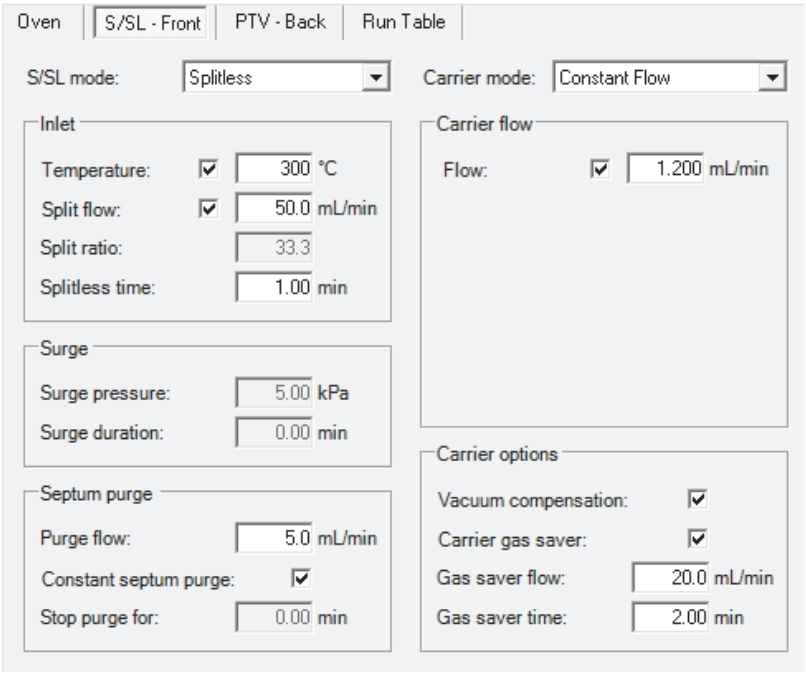

Figure 2. TRACE 1310 GC method setup SSL injector

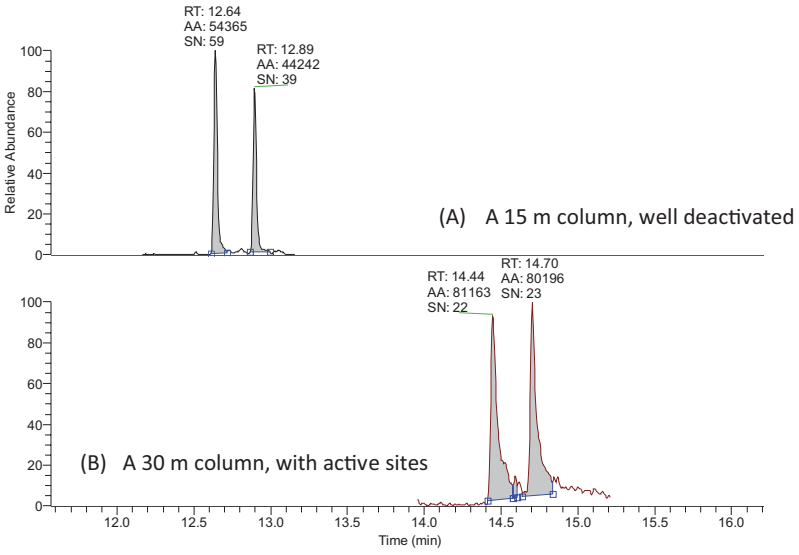

Figure 3. Capsaicin and dihydrocapsacin elution, (A) from a short 15 m well deactivated capillary column at 100 ppb, (B) from a 30 m column with active sites at 500 ppb, resulting in poor peak shape and low S/N value. Both column dimensions are 0.25 µm film thickness, 0.25 mm ID, and no analyte protectant was added.

Sample Measurements

The active compounds capsaicin and dihydrocapsacin elute with only a short retention time difference. A good separation free from peak tailing is necessary for a reliable peak integration for low RSD values at low concentration levels (Figure 3). It was found with different types of GC columns that the quality of the column deactivation, age of the column and matrix deposits have a detrimental effect on the capsaicin and dihydrocapsacin peak shape and quantitative reproducibility. Also, piperine was affected while thymol always showed symmetrical peak shapes, apparently being unaffected by the increasingly active column film conditions.

To preserve inert conditions with the inlet liner and analytical column for high quantitative precision and reproducible results with a high number of samples, an analyte protectant was co-injected with the extract of active analytes [4, 5, 6]. These compounds are known to be used in pesticides analysis, also comprising a number of active and polar compounds. A concentration of 2 ppm of sorbitol was added to the extracts in all experiments.

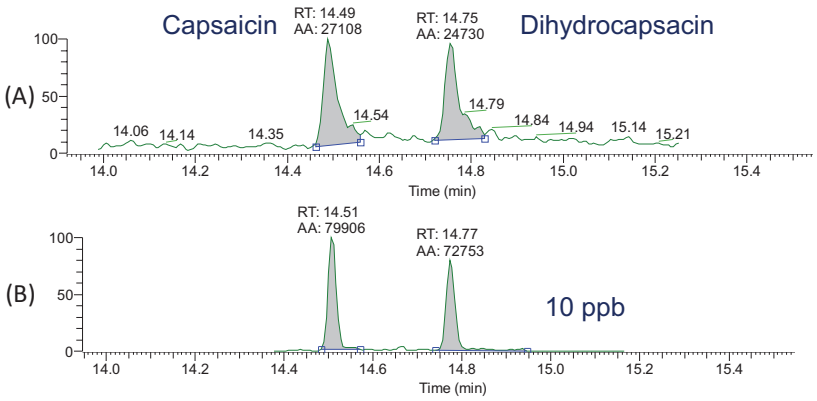

Figure 4. Capsaicin and dihydrocapsacin peak shape, (A) without and (B) with analyte protectant, both runs at 10 ppb concentration, 30 m column of Figure. 3.

Results

All measurements were carried out using the above described instrumental setup with a co-injection of sorbitol as analyte protectant. Symmetrical peak shapes for all compounds of interest, including the critical pair capsaicin and dihydrocapsacin, could be achieved, as shown in Figure 4. The individual peaks for selected compounds of the calibration runs, normalized to 100% each, are given in Figures 5-8. The linear quantitative calibrations with a zoom into the low concentration range of 10-200 ppb are shown in Figure 9.

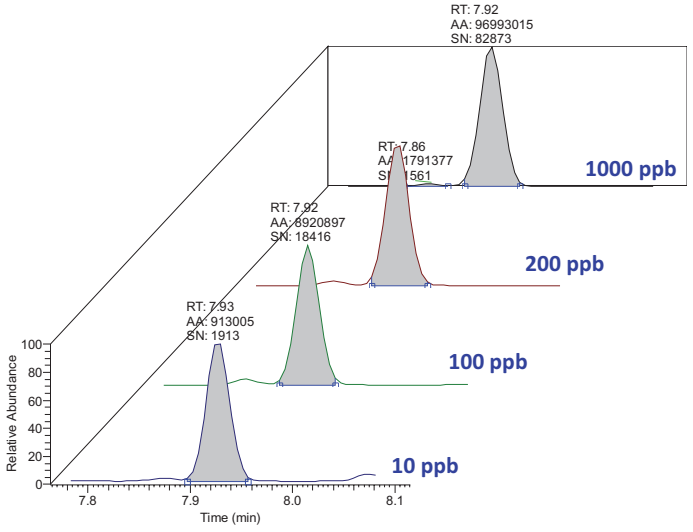

Figure 5. Thymol calibration peaks 10-1000 ppb.

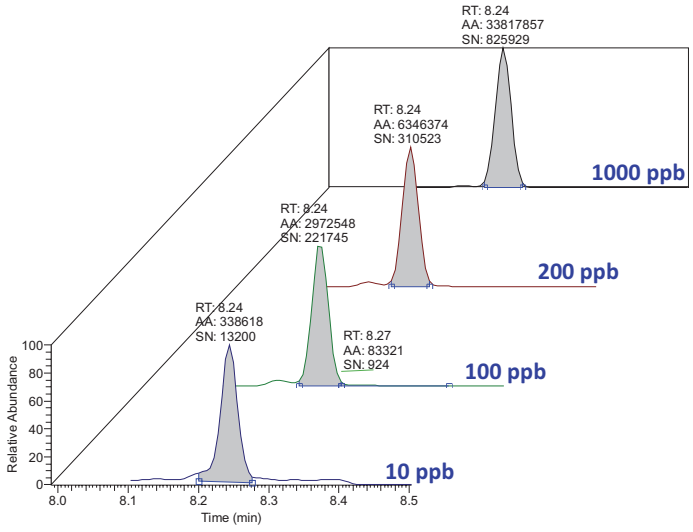

Figure 6. α-Methyl-trans-cinnamaldehyde calibration peaks 10-1000 ppb.

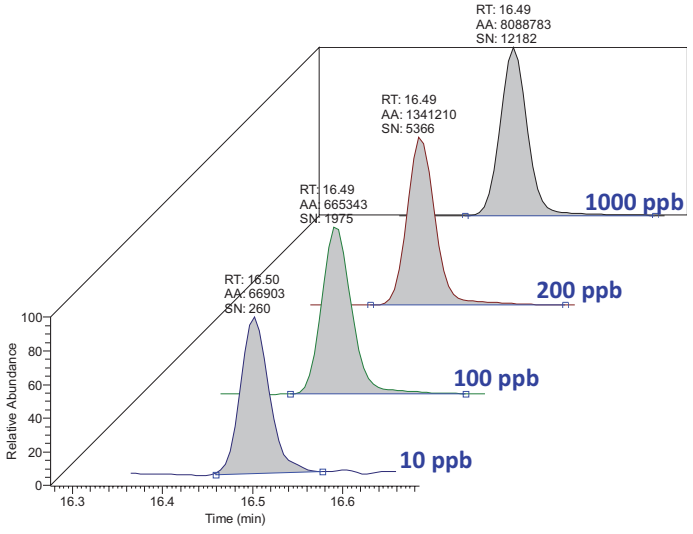

Figure 8. Piperine calibration peaks 10-1000 ppb.

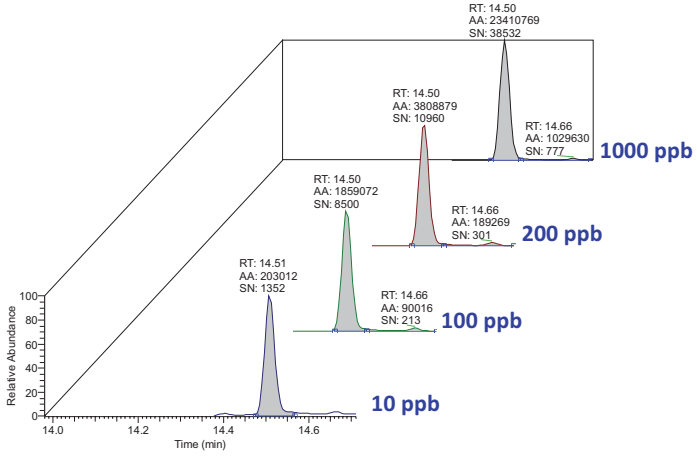

Figure. 7. Capsaicin calibration peaks 10-1000 ppb.

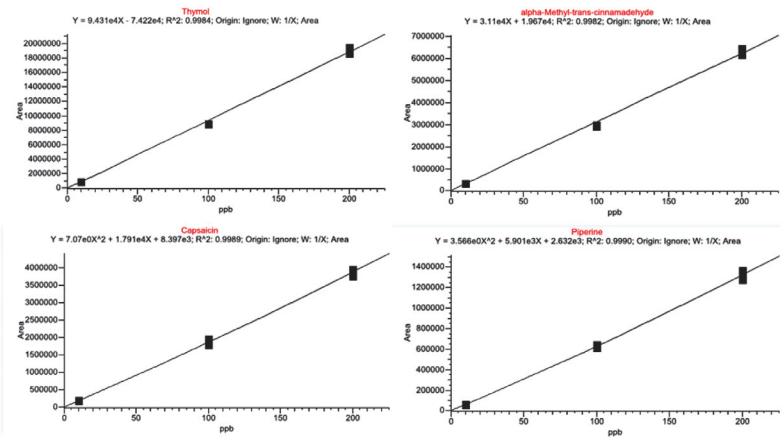

Figure 9. Quantitative calibrations on low concentration side 10-200 ppb.

Table 3. Precision of a spiked spice sample analysis

Compound Name	Day 1 [area cts]	Day 2 [area cts]	Day 3 [area cts]	RSD%
Thymol	96.513	94.128	91.462	2.70%
alpha-Methyl-trans-cinnamadehyde	97.665	93.579	92.918	2.70%
Capsaicin	100.669	105.363	99.392	3.10%
Dihydrocapsacin	102.752	103.852	101.089	1.40%
Piperine	104.307	106.685	103.274	1.70%

Precision

For a reproducibility study, a series of three measurements on three consecutive days was performed, and the precision of the area results calculated as relative standard deviation (RSD %). The peak area precision for thymol, α-methyl-trans-cinnamadehyde, capsaicin, and piperidine was determined for the low level calibration points up to 200 ppb. Thymol shows values in all cases of 3% RSD and below. This compound is less affected by potential active sites in the GC system. The active compounds α-methyl-trans-cinnamaldehyde, capsaicin, and piperidine also show excellent precision data ranging from 0.5% to 8% RSD over the length of the study. This excellent area precision of the other active analytes at low concentration levels is achieved by the use of an analyte protectant in the applied extracts.

A spiked real life spice sample has been measured on three consecutive days, as well, to calculate the precision of the measurements. The peak area data in Table 3 indicate a low level spike below 10 ppb. The reproducibility over three days for all compounds tested is in the range of 1-3%.

Conclusions

The described method using the TSQ 8000 GC-MS/MS system provides a very sensitive and precise assay for the trace analysis of receptor-sensitizing and active compounds like capsaicin, piperine, and thymol. Excellent symmetrical and stable peak shape can be achieved for these polar spice components by using sorbitol as analyte protectant.

Analyte protectants can reduce the phenomenon of poor chromatographic peak shapes and keep the chromatographic integrity over long sample series with symmetrical peaks and very stable results with excellent precision. Automatic peak integration is facilitated, peak areas are increased, and the reproducibility improved significantly.

All the investigated compounds, in particular capsaicin, piperine, and thymol, can be detected with high signal-to-noise ratio even at the low 10 ppb level. A matrix sample with measured concentrations well below 10 ppb demonstrated the excellent reproducibility of the TRACE 1310 GC system. The precision in all measured levels including the low 10 ppb concentration was below 10% RSD.

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Application Note 20733

A GC-FID Method for the Comparison of Acid- and Base-Catalyzed Derivatization of Fatty Acids to FAMEs in Three Edible Oils

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Key Words

TR-FAME, fatty acid methyl esters (FAMEs), BF_3-methanol, derivatization, *cis*- and *trans*-fatty acid

Abstract

This application note demonstrates the analysis of 37 fatty acid methyl esters (FAMEs) separated by a highly polar phased Thermo Scientific™ TRACE™ TR-FAME GC column. Results from two derivatization methods (acid and base esterification) were compared for their efficiency in converting fatty acids to their methyl esters on three different fat matrices prior to GC analysis.

Introduction

Gas chromatography is the preferred analytical method for the determination of fatty acid methyl esters (FAMEs). The fatty acid content of food was analyzed after derivatization to their methyl ester products. This conversion involved either an acid or base esterification process.

In this application, separation of a mixture of 37 FAMEs in a reference standard was achieved on a TRACE TR-FAME 100 m × 0.25 mm × 0.2 µm GC column. The reference standard contained a wide range of carbon chain lengths (C4–C24), with concentrations between 2–6% wt/wt. The high polarity phase GC column is optimized for separating complex mixtures of *cis*- and *trans*-fatty acids.

The base esterification method [1] was used to derivatize the fatty acid content in three fat matrices (palm oil, margarine, butter) and this was compared with acid catalyzed esterification [2] under equivalent conditions. The FAME components in the three fat matrices were then identified using the retention times established using a 37 component reference standard.

BF_3-methanol is one of the fastest and most convenient ways to convert fatty acids to their methyl ester derivatives. The reagent is supplied in an easy-to-use, septum-sealed Hypo vial and offers convenient syringe removal of the reagent without exposing it to air. Use of the BF_3-methanol reagent results in improved detection of fatty acids in a fatty food matrix while maintaining good chromatographic peak shape.

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Experimental Details

Consumables		Part Number
Column:	TRACE TR-FAME GC column, 100 m × 0.25 mm × 0.20 µm	260M238P
Septum:	Thermo Scientific BTO, 17 mm	31303211
Liner:	Thermo Scientific™ Split FocusLiner™, 3 × 8 × 105 mm	45350031
Column ferrules:	100% graphite ferrules for Thermo Scientific™ TRACE™ injector 0.1–0.25 mm i.d.	29053488
Column ferrules:	Graphite/Vespel® for transfer line 0.1–0.25 mm i.d.	29033496
Injection syringe:	10 µL fixed needle syringe for Thermo Scientific™ TriPlus™ Autosampler	36500525
Vials and closures:	Thermo Scientific™ Chromacol™ 9 mm screw 0.3 mL fixed insert amber Micro+ vials	03-FISV (A)
	Chromacol 9 mm screw caps with Silicone/PTFE septa	9-SC(B)-ST101
Syringes:	Thermo Scientific™ National™ 30 mm GMF Syringe filter membrane, 3.1 µm pore size	F2500-20
	Thermo Scientific™ National™ Target™ 3 mL plastic disposable syringes	S7510-3

Sample Handling Equipment	Part Number
Thermo Scientific™ Reacti-Therm™ III Heating/Stirring Module	TS-18823
Thermo Scientific™ Reacti-Vap™ III Evaporator	TS-18826
Thermo Scientific Reacti-Vap Block	TS-18814
Thermo Scientific™ Reacti-Vial™ Reaction Vials 10 mL	TS-13225

Chemicals and Reagents	Part Number
Fisher Scientific™ HPLC grade hexane	H/0403/15
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade methanol	M/4056/17
Fisher Scientific HPLC grade potassium hydroxide	S/9220/PB08
Thermo Scientific 14% BF_3-methanol esterification reagent	TS-49370

Sample Preparation

Butter, margarine, and palm oil were treated by two derivatization methods. In the first method, potassium hydroxide / methanol was used to esterify the fat samples [1]. This was then compared to an acidic derivatization method involving BF_3-methanol [2].

Base esterification:	A 50 mg liquid fat sample was weighed into Reacti-Vial containing a magnetic stirrer and 1 mL of hexane and 2 mL of 4 mol/L potassium hydroxide / methanol were added. The Reacti-Vial was capped and placed in the Reacti-Therm module for 30 minutes at 50 °C. The mixture was cooled to room temperature and 1 mL of water was then added. After phase separation, an aliquot of the organic layer was transferred to a fixed insert GC vial.
Acid esterification:	A 50 mg liquid fat sample was weighed into a Reacti-Vial containing a magnetic stirrer and 1 mL of hexane and 0.5 mL of Thermo Scientific 14% BF_3-methanol was added. The Reacti-Vial was capped and placed in the Reacti-Therm module for 30 minutes at 50 °C. The mixture was cooled to room temperature and 1 mL of water was then added. After phase separation, an aliquot of the organic layer was transferred to a fixed insert GC vial.

Separation Conditions	
Instrumentation:	Thermo Scientific™ TRACE™ GC Ultra Gas Chromatograph
Carrier gas:	Helium
Split flow:	10 mL/min
Split ratio:	10:1
Column flow:	1.0 mL/min, constant flow
Oven temperature:	100 °C (0.2 min), 2 °C/min, 240 °C (15 min)
Injector type:	Split/Splitless
Injector mode:	Split, constant septum purge
Injector temperature:	240 °C
Detector type:	Flame ionization detector (FID)
Detector temperature:	250 °C
Detector air flow:	350 mL/min
Detector hydrogen flow:	35 mL/min
Detector nitrogen flow:	30 mL/min

Injection Conditions	
Instrumentation:	TriPlus Autosampler
Injection volume:	1 µL

Results

The analysis of a 37 component FAME reference standard was successfully carried out using a TR-FAME GC column (Figure 1). The high polarity phase on the TRACE TR-FAME GC column provided baseline resolution of the majority of FAME components, apart from C20:3 [*cis*-8, 11, 14], C22:1 [*cis*-13], and C20:3 [*cis*-8, 14, 17], which were partially separated. These compounds, two of which are isomeric, are known to be difficult to separate by GC due to their structural similarities, which results in poor resolution. All FAME components exhibited excellent chromatographic peak shape.

A qualitative analysis was performed by comparing the FAME peaks in the fat matrices using the two derivatization methods. The components were identified using the retention times in the FAME reference standard in Figure 1. The results from the two methods are compared in Figures 2 and 3, under equivalent conditions (see Table 2 for comparison).

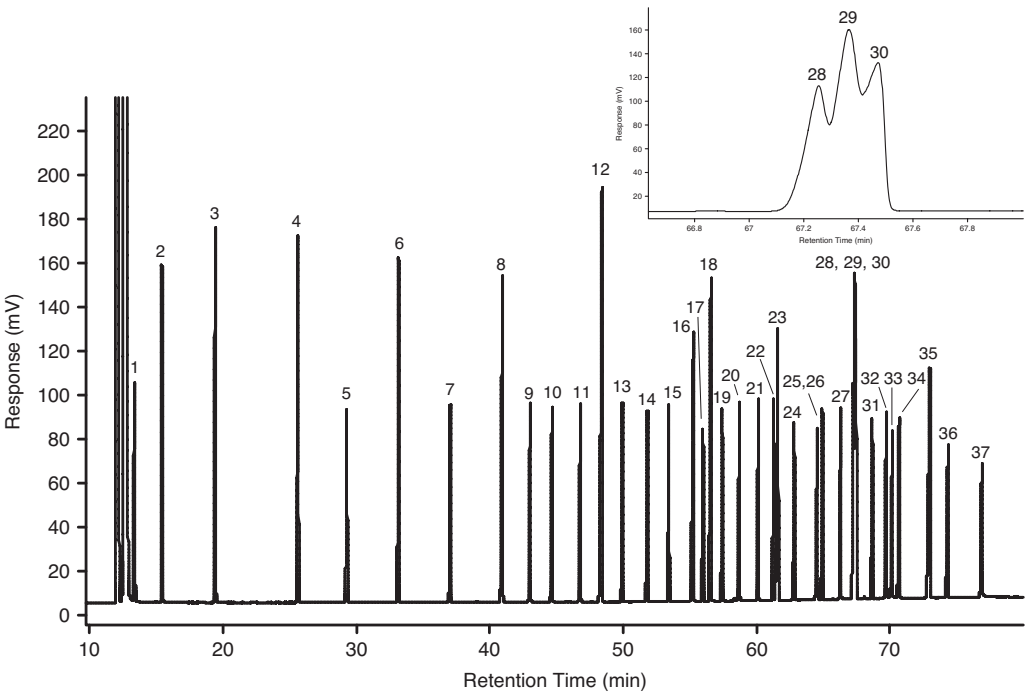

Figure 1: Chromatogram of 37 components FAME mixture (reference standard) separated on a TR-FAME 100 m × 0.25 mm × 0.20 µm GC column

Elution order	Compound	Concentration %wt/wt	t_R/min
1	Methyl butyrate (C4:0)	4	13.40
2	Methyl caproate (C6:0)	4	15.53
3	Methyl capylate (C8:0)	4	19.60
4	Methyl decanoate (C10:0)	4	25.90
5	Methyl undecanoate (C11:0)	2	29.56
6	Methyl dodecanoate (C12:0)	4	33.50
7	Methyl tridecanoate (C13:0)	2	37.36
8	Methyl myristate (C14:0)	4	41.30
9	Methyl myristoleate (C14:1 [*cis*-9])	2	43.36
10	Methyl pentadecanoate (C15:0)	2	45.01
11	Methyl pentadenoate (C15:1 [*cis*-10])	2	47.12
12	Methyl palmitate (C16:0)	6	48.79
13	Methyl palmitoleate (C16:1 [*cis*-9])	2	50.30
14	Methyl heptadecanoate (C17:0)	2	52.18
15	Methyl heptadenoate (C17:1 [*cis*-10])	2	53.78
16	Methyl stearate (C18:0)	4	55.67
17	Methyl octadecenoate (C18:1 [*trans*-9])	2	56.38
18	Methyl oleate (C18:1 [*cis*-9])	4	56.96
19	Methyl linoleaidate(C18:2 [*trans*-9,12])	2	57.79
20	Methyl linoleate (C18:2 [*cis*-9,12])	2	59.06
21	Methyl arachidate (C20:0)	4	60.48
22	Methyl linolenate (C18:3 [*cis*-6,9,12])	2	61.61
23	Methyl (C20:1 [*cis*-11])	2	62.01
24	Methyl linolenate (C18:3 [*cis*-9,12,15])	2	63.20
25	Methyl heneicosanoate(C21:0)	2	64.96
26	Methyl eicosadienoate (C20:2 [*cis*-11,14])	2	65.31
27	Methyl behenate (C22:0 FAME)	4	66.66
28	Methyl eicosatrienoate (C20:3 [*cis*-8,11,14)	2	67.60
29	Methyl erucate (C22:1 [*cis*-13])	2	67.72
30	Methyl eicosatrienoate (C20:3 [*cis*-11,14,17])	2	67.87
31	Methyl arachidonate (C20:4 [*cis*-5,8,77,14])	2	69.06
32	Methyl tricosanoate (C23:0)	2	70.06
33	Methyl docosadienoate (C22:2 [*cis*-13,16])	2	70.57
34	Methyl lignocerate (C24:0)	4	71.06
35	Methyl *cis*-5,8,11,14,17-eicosapentaenoate	2	73.41
36	Methyl nervonate (C24:1 [*cis*-15])	2	74.74
37	Methyl *cis*-4,7,10,13,16-docosahexenoate	2	77.23

Table 1: FAMEs according to the elution order and retention times for the reference standard

The base esterification process resulted in fewer FAME peaks observed in the fat samples compared to the acid esterification process (see Table 2 for comparison). Some emulsification occurred during phase separation and the esterified solution required filtering with a syringe filter prior to GC analysis.

In contrast, acidic esterification produced more FAME peaks than base esterification. The strong Lewis acid BF_3-methanol can more efficiently esterify fatty acids compared with the base esterification method, with no white emulsion appearing when reaction is worked up with water.

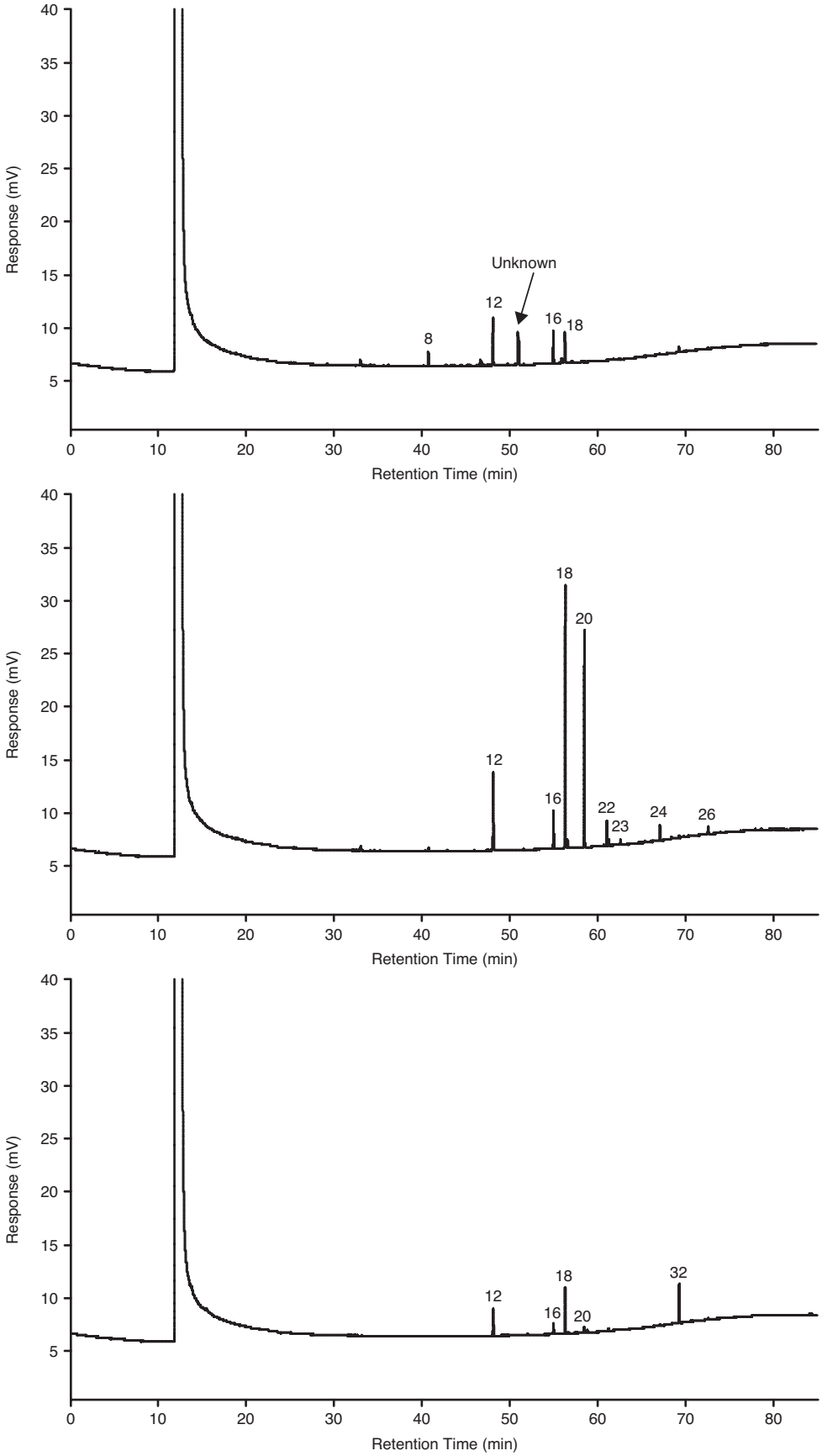

Figure 2: Chromatograms of (top) butter, (middle) margarine, and (bottom) palm oil sample derivatized by potassium hydroxide / methanol

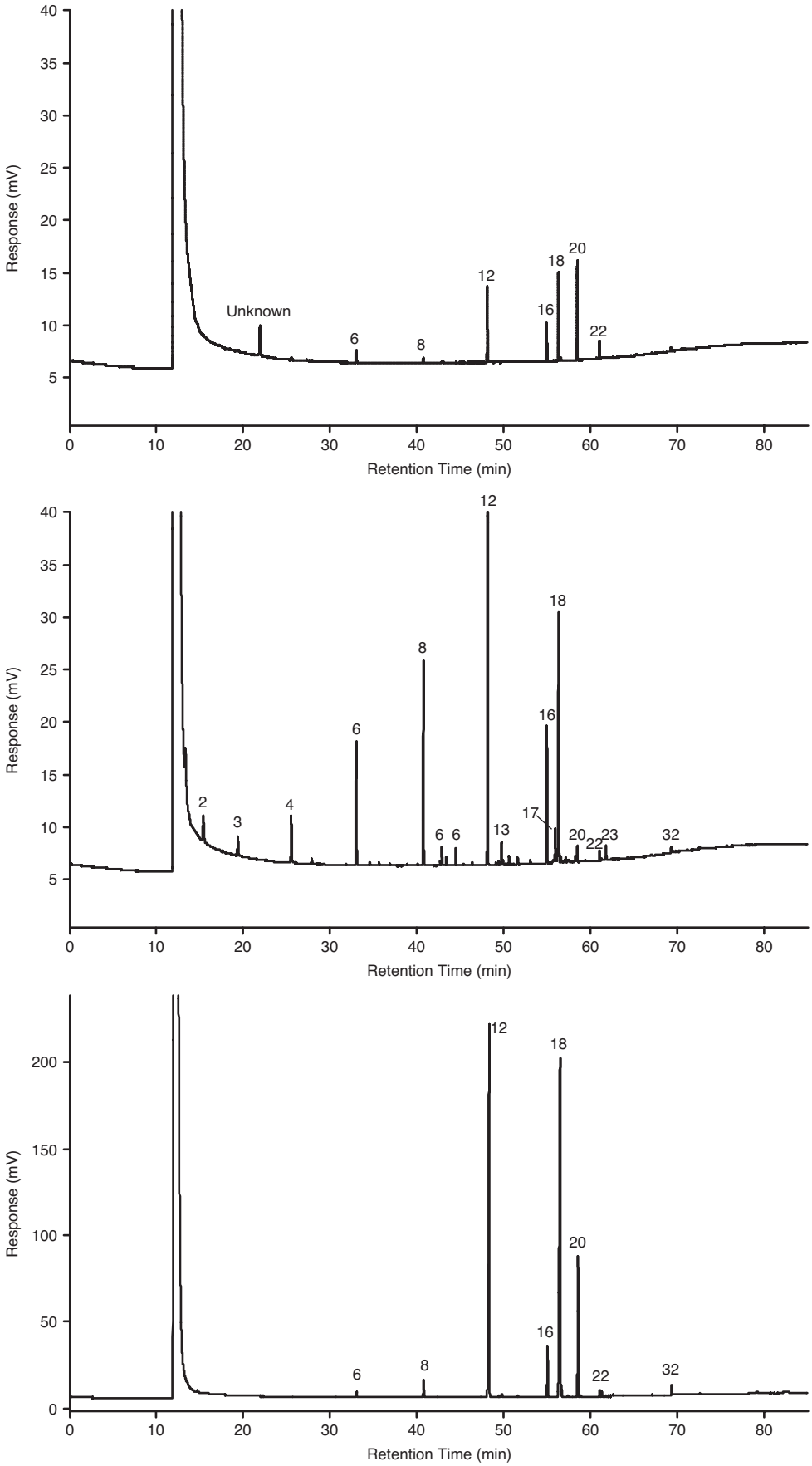

Figure 3: Chromatograms of (top) butter, (middle) margarine, and (bottom) palm oil sample derivatized by Thermo Scientific Reagent BF_3-methanol

Matrix	Base esterified-FAMEs using KOH / methanol (Figure 2)	Acid esterified- FAMEs using BF_3-methanol (Figure 3)
Butter	-	(6) C12:0
	(8) C14:0	(8) C14:0
	(12) C16:0	(12) C16:0
	(16) C18:0	(16) C18:0
	(18) C18:1 [*cis*-9]	(18) C18:1 [*cis*-9]
	-	(20) C18:2 [*cis*-9,12]
	-	(22) C18:3 [*cis*-6,9,12]
Margarine	-	(2) C6:0
	-	(3) C8:0
	-	(4) C10:0
	-	(6) C12:0
	-	(8) C14:0
	-	(9) C14:1 [*cis*-9]
	-	(10) C15:0
	(12) C16:0	(12) C16:0
	-	(13) C16:1 [*cis*-9]
	(16) C18:0	(16) C18:0
	-	(17) C18:1 [*trans*-9]
	(18) C18:1 [*cis*-9]	(18) C18:1 [*cis*-9]
	(20) C18:2 [*cis*-9,12]	(20) C18:2 [*cis*-9,12]
	(22) C18:3 [*cis*-6,9,12]	(22) C18:3 [*cis*-6,9,12]
	(23) C20:1 [*cis*-11]	(23) C20:1 [*cis*-11]
	(24) C18:3 [*cis*-9,12,15]	-
	(32) C23:0	(32) C23:0
Palm oil	-	(6) C12:0
	-	(8) C14:0
	(12) C16:0	(12) C16:0
	(16) C18:0	(16) C18:0
	(18) C18:1 [*cis*-9]	(18) C18:1 [*cis*-9]
	(20) C18:2 [*cis*-9,12]	(20) C18:2 [*cis*-9,12]
	-	(22) C18:3 [*cis*-6,9,12]
	(32) C23:0	(32) C23:0

Table 2: FAMEs peaks observed for two derivatization methods in 30 minutes reaction time

Conclusion

The Thermo Scientific reagent BF_3-methanol provides a fast and efficient way of converting fatty acids to their methyl esters in fat samples. The TRACE TR-FAME GC column can separate a complex mixture of 37 FAMEs with excellent peak shapes.

References

[1] Chinese Official method SN/T 1945-2007. Determination of fatty acids in food-Capillary gas chromatography

[2] Thermo Scientific Reagents, Solvents and Accessories Brochure. Ref BR20535_E 06/12S

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Analysis of Acrylamide in Potato Chips by SPE and GC-MS

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Key Words

Hypercarb SPE, food, acrylamide, 2-propenamide, capillary GC, porous graphic carbon (PGC), polyethylene glycol (PEG) GC column

Abstract

Acrylamide is an endogenous compound, formed when heating starchy or sugary foods. The production of potato chips can result in its formation. The method reported here detects acrylamide at the low ng/g levels at which it is produced. Potato chips were extracted using porous graphitic carbon for solid phase extraction (SPE). Analysis of acrylamide was performed using GC-MS on a polyethylene glycol phase GC column. A standard addition calibration curve was used to estimate the level of acrylamide in potato chips at 450 ng/g.

Introduction

Acrylamide (2-propenamide) is a potential human carcinogen. This toxic compound is usually formed as a by-product of Maillard reactions during the heating of carbohydrate-rich food. The World Health Organization (WHO) has set a safe limit of 500 ng/mL acrylamide in drinking water. Higher levels of 100–1000 ng/g are determined in some foods such as potato chips or french fries.

The extraction of acrylamide from potato chips is carried out using a Thermo Scientific™ HyperSep™ Hypercarb™ SPE cartridge. Hypercarb SPE material is 100% porous graphic carbon (PGC) and offers retention of highly polar compounds that are not usually retained by traditional reversed phase C18 columns. HyperSep Hypercarb SPE can produce clean samples by removing potential matrix interferences.

The analysis of acrylamide was carried out using a GC-MS in electron ionization (EI) mode. Quantitative measurement in food can be difficult as matrix-derived ions can interfere with acrylamide fragment ions of *m/z* 71, 55, and 41 when using this mode. Acrylamide often requires derivatization to improve sensitivity on a mass spectrometer. In this case, acrylamide is injected without derivatization onto a Thermo Scientific™ DSQ™ II mass spectrometer and an ultra low bleed Thermo Scientific™ TraceGOLD™ TG-WaxMS™ 30 m × 0.25 mm × 0.25 µm GC column.

Acrylamide is a highly polar water soluble compound having a logP value of -0.65 [1]. Such highly polar compounds are not readily amenable to GC, therefore a polar GC column is required. The TraceGOLD TG-WaxMS column is a polyethylene glycol-phase GC column that allows the analysis of polar compounds.

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Experimental Details

Consumables		Part Number
Cartridge type:	HyperSep Hypercarb SPE cartridge, 500 mg/6 mL	60106-402
Column:	TraceGOLD TG-WaxMS, 30 m × 0.25 mm × 0.25 µm	26088-1420
Septum:	Thermo Scientific BTO, 17 mm	31303211
Liner:	Thermo Scientific™ Splitless FocusLiner™, 3 × 8 × 105 mm	45354032
Column ferrules:	100% graphite ferrules for Thermo Scientific™ TRACE™ injector, 0.1–0.25 mm i.d.	29053488
Column ferrules:	Graphite/Vespel® for transfer line 0.1–0.25 mm i.d.	29033496
Vials and closures:	Thermo Scientific™ Chromacol™ 9 mm screw, 2 mL vial, amber	2-SVW(A)
	Chromacol 9 mm screw caps with silicone/PTFE septa	9-SC(B)-ST101
Syringe filter:	Thermo Scientific™ Target2™ 30 mm GMF syringe filter membrane, 3.1 µm pore size	F2500-20
Plastic syringe:	Thermo Scientific 3 mL plastic disposable syringes	S7510-3

Sample Handling Equipment	Part Number
HyperSep glass block manifold	60104-232

Instrumentation
Thermo Scientific™ TRACE GC Ultra™ gas chromatograph
Thermo Scientific™ DSQ™ II single quadrupole mass spectrometer
Thermo Scientific™ TriPlus™ Autosampler

Chemicals and Reagents	Part Number
Fisher Scientific™ HPLC grade water	W/0106/17
Fisher Scientific HPLC grade methanol	M/4056/17
Fisher Scientific Analytical grade formic acid	F/1900/PB08

Sample Pretreatment

The potato chips were finely crushed with mortar and pestle and 1 g was weighed into a vial. A 1 g portion of the sample was spiked with 25, 50, 100, 250, 500, and 1000 ng/g of acrylamide standard in 2% formic acid / water. The sample was then filtered through a filter membrane.

Sample Preparation	
Compounds:	Acrylamide and acrylamide-d_3 (internal standard)
Matrix:	Potato chips
Conditioning stage:	Add 4 mL methanol, 4 mL water, and 4 mL 2% formic acid / water to the SPE cartridge.
Application stage:	Apply 1 mL of extract in 2% formic acid / water under vacuum at 1 mL/min to the SPE cartridge.
Washing stage:	Add 1 mL water to the SPE cartridge and dry for 20 min under vacuum.
Elution stage:	Apply 4 mL methanol to the SPE cartridge.
Additional stage:	Evaporate methanolic extract and reconstitute with 1 mL of 1 µg/mL of internal standard in methanol to the SPE cartridge.

Separation Conditions	
Carrier gas:	Helium
Split flow:	50 mL/min
Column flow:	1.2 mL/min, constant flow
Oven temperature:	80 °C, 10 °C/min, 250 °C
Injector type:	Split/Splitless
Injector mode:	Splitless (1 min), constant septum purge
Injector temperature:	230 °C

Instrumentation	
Transfer line temperature:	150 °C
Source temperature:	200 °C
Ionization conditions:	EI
Electron energy:	70 eV
SIM scan parameters:	*m/z* 71 for acrylamide and *m/z* 74 for acrylamide-d_3
Start time:	4.0 min
Dwell time:	0.1 s

Injection Conditions	
Injection volume:	2 µL
Pre- and post-needle injection dwell time:	0.5 s

Data Processing	
Software:	Thermo Scientific™ Xcalibur™ software

Results

A standard addition calibration curve was constructed for acrylamide in matrix over the range 25–1000 ng/g. Standard addition calibration was chosen because acrylamide is endogenous in cooked foods and a suitable blank matrix was unavailable.

The amount of acrylamide present in the potato chips was calculated to be 450 ng/g. The chromatogram in Figure 1 shows the acrylamide peak in potato chips and acrylamide-d_3 internal standard spiked in potato chips.

The acrylamide concentration was calculated using the integrated response ratio of acrylamide/acrylamide-d_3 (*m/z* 71/74). The acrylamide in the potato chips was calculated from the intercept of the x axis. An excellent linearity was demonstrated for this method with a coefficient of determination (R^2) of 0.999.

The accuracy of the back calculated concentrations for the amount of acrylamide spiked in potato chips was less than 10% (see Table 1).

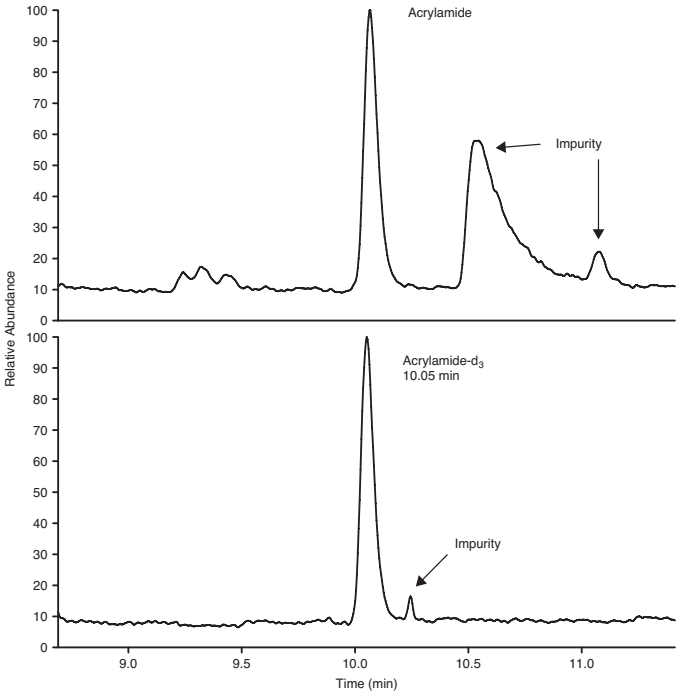

Figure 1: TIC of chromatogram of 1 µg/mL spiked acrylamide (*m/z* 71) and acrylamide-d_3 (*m/z* 74) extracted from potato chips

Specified Concentration (µg/mL)	Calculated Concentration	% Difference
0.25	0.225	-9.83
0.50	0.469	-6.22
1.00	0.983	-1.70
2.50	2.520	0.79
5.00	4.979	-0.41
10.0	10.037	0.37

Table 1: Accuracy data for the standard addition calibration curve for spiked acrylamide in potato chips

Conclusion

HyperSep Hypercarb SPE cartridges offer high levels of reproducibility as well as cleaner extracts, which yields very good results. TraceGOLD TG-WaxMS GC columns are suitable for the GC-MS analysis of acrylamide because of the low bleed stationary phase and better retention of polar analytes compared with lower polarity stationary phases.

Reference

[1] Acrylamide in Drinking-water - Background document for development of WHO Guidelines for Drinking-water, 2011, http://www.who.int/water_sanitation_health/dwq/chemicals/acrylamide.pdf

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Application Note: 52242

Identification and Quantification of Impurities in Wines by GC/MS

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(With special thanks to members of Œnologic Center of Grezillac)

Key Words

- ISQ Single Quadrupole GC-MS
- TRACE GC Ultra
- Food and Beverage
- SPME
- Wine

Introduction

While wine makers have historically used gas chromatography and mass spectrometry (GC/MS) to detect pesticides, they now more commonly use the technique to supplement quality control checks of wine taste. Without GC/MS, wine makers must rely on expert evaluation by oenologists to determine wine quality. By identifying maturation tracers and molecules commonly responsible for taste defects, GC/MS augments expert opinion with objective and quantitative information. When using a SPME extraction method, GC/MS has the additional advantages of requiring very small sample sizes, a minimum of sample preparation, and rapid analysis of target molecules.

Several types of molecules, while not dangerous to humans, affect wine taste and quality, such as volatile phenol compounds derived from *Brettanomyces* yeast metabolism.[1,2] Haloanisoles such as 2,4,6-tricholoranisole that result from cork fungal infections also affect wine taste.[3,4] Methoxypyrazines such as 3-isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) are maturation markers, and detecting their levels can help determine ideal grape harvest time.[5] An automated technique with repeatable results for detecting these compounds is highly desirable, and GC/MS can provide such a method.

Extracted wine samples were analyzed by a sequential full-scan/SIM acquisition on a GC-MS system consisting of a Thermo Scientific ISQ single-quadrupole mass spectrometer and a Thermo Scientific TRACE GC Ultra gas chromatograph. The results were compared to the sensitivity limits of human tasters. This method allows wine makers to obtain precise measurements on the organoleptic parameters that determine wine purity on site rather than having to send samples for expensive, external analysis. In this report, we present the design and results of this study, including the experimental method used to detect impurities and the concentration ranges that compare GC/MS with human detection.

Figure 1: ISQ Single Quadrupole GC-MS system

Methods

For this experiment, several targeted molecule types that affect wine quality were analyzed using an ISQ™ Single Quadrupole GC-MS system (Figure 1). Table 1 contains a brief description of the effects on wine quality of the four target molecule types, and examples of how GC/MS analysis can provide value in quality control.

Molecule Type	Description of Effect on Wine	Benefit of GC/MS Analysis
Volatile Phenols (4-ethylphenol, 4-ethylgaiacol, 4-vinylphenol, 4-vinylgaiacol)	Volatile phenols are produced in various steps of *Brettanomyces* yeast metabolism. The two produced in the final step – 4-ethylphenol and 4-ethylgaiacol – give the wine an "animal" taste and depreciates its quality.	GC/MS can detect 4-ethylphenol and 4-ethylgaiacol in lower concentration than human tasters. GC/MS can also detect the presence of 4-vinylphenol and 4-vinylgaiacol, intermediaries in *Brettanomyces* yeast metabolism and allow wine makers to discard contaminated batches.
Geosmine	This fragrant compound derived from moldy grapes interferes with a wine's taste.	Detecting geosmine in wine alerts makers to the presence of mold in their grapes and allows them to locate and treat a contaminated plot of land.
Haloanisoles (TBA, TCA, TeBA, PCA)	These compounds come from halophenols, compounds used to prevent wood degradation in vines. They give wine a moldy odor.	Assays provide information of an organoleptic default in wine production and help identify contamination sources.
Methoxypyranzines (IBMP, IPMP)	IBMP and IPMP are maturation markers, and their levels decrease as wine matures. IBMP gives wine a "green pepper" taste; IPMP imparts an earthy flavor.	Determining the levels of IBMP and IPMP in wine affects harvesting decisions.

Table 1: Targeted molecules affecting wine purity

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Sample Preparation

To prepare the samples, a 10 mL sample of wine was saturated with NaCl. The sample was placed in a vial and extracted using SPME. A PDMS/DVB 65 µm StableFlex™ SPME Fiber (SUPELCO-57293U) was used, and the fiber was exposed to the sample for agitation for 30 minutes at 70 °C at three-second intervals.

Instrumental Analysis

The ISQ mass spectrometer used for this analysis was set to perform sequential full scan/SIM acquisitions. The TRACE™ GC Ultra was equipped with a standard split/splitless injector. The split/splitless injector temperature was set to 220 °C, and a splitless injection was used. The ISQ GC-MS parameters are summarized in Table 2. The analytical column used was a Thermo Scientific TraceGOLD TG-5MS 15 m × 0.25 mm i.d. × 0.25 µm film (PN 26098-1300). TCA d5 was used as an internal standard; its SIM ions are 215 and 217.

The results were analyzed using Thermo Scientific QuanLab Forms software. QuanLab™ Forms automatically tests the expected retention times (RT), actual ratio versus range of tolerance, and the coelution of ions. QuanLab Forms is also Directorate-General for Health and Consumer Protection (SANCO) compliant and can be used in the European Union.

Results

The spectra of the sequential SIM scan can be seen in Figure 2. The SIM ions monitored using the ISQ are listed in Table 3. Figures 3 through 7 present the calibration curves of several of the target molecules at various linearity ranges. Calibration ranges were established according to the range of human perception – and to the range of interest for oenologists – as opposed to instrument performance.

For all these target molecules, the GC-MS was able to detect lower concentrations than the limits of human perception.

ISQ	
Source Temp (°C)	200
Detector Gain	1×10^5
Start Time (min)	0.2
Acquisition End Time (min)	40
Full Scan Range (u)	35–450
Dwell Time (ms)	20
SIM Ions	See Table 3

TRACE GC Ultra	
Oven Method	
Initial Temp (°C)	40
Initial Time (min)	1.0
Rate #1 (°C/min)	5
Initial Temp #2 (°C)	60
Initial Time #2 (min)	1
Rate #2 (°C/min)	3
Initial Temp #3 (°C)	125
Hold Time #3 (min)	1
Rate #3 (°C/min)	10
Final Temp (°C)	238
SSL Method	Splitless
Temperature (°C)	220
Mode	Splitless
Splitless Time	3 min
Carrier Flow (mL/min)	1.2
Gas Saver	On
Vacuum Compensation	On
Transfer Line (°C)	250

Table 2: Instrument method summary for the full scan/SIM analysis of target molecules on the ISQ and TRACE GC Ultra

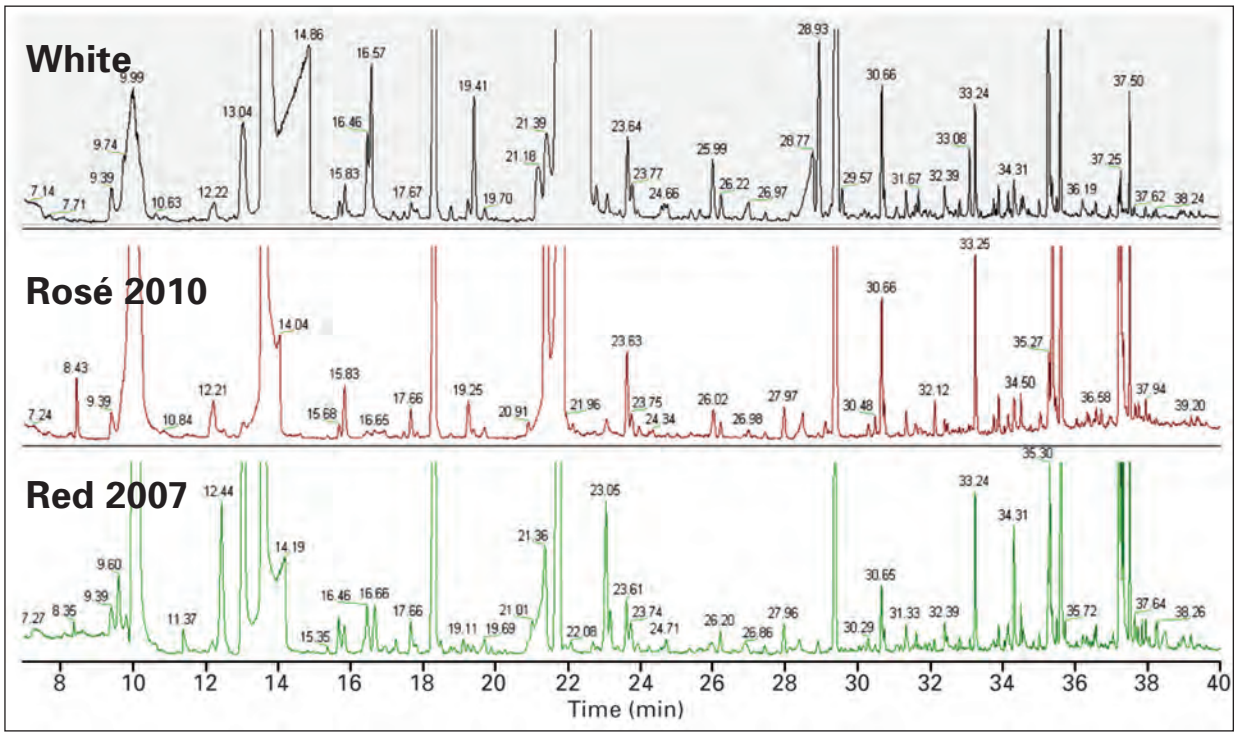

Figure 2: Chromatograms showing full-scan acquisitions for three wine types

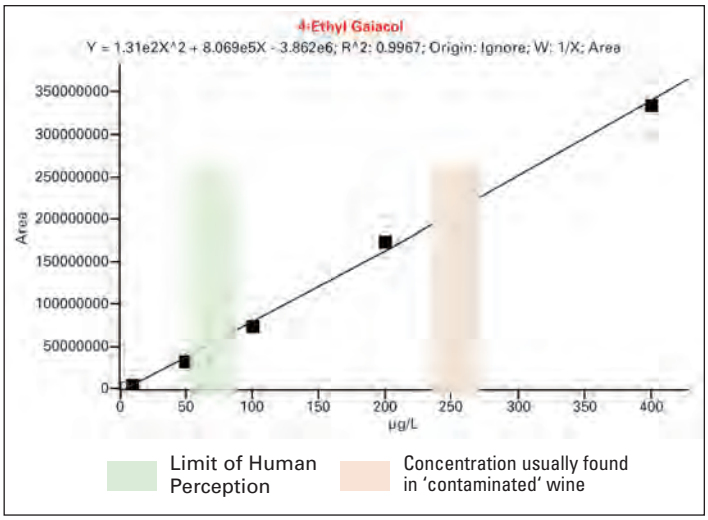

Figure 3: 4-Ethylgaiacol from 50 to 100 µg/L

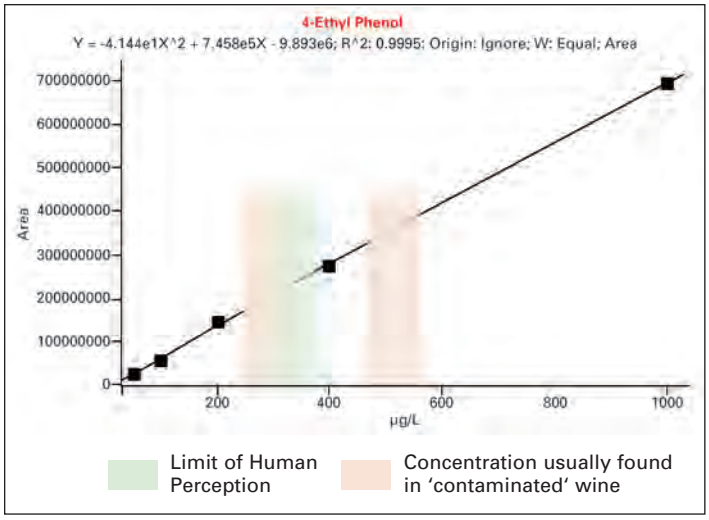

Figure 4: 4-Ethylphenol from 300 to 400 µg/L

Target Molecule	*m/z*
IPMP	124, 137, 152
IBMP	94, 124, 151
4-Ethylphenol	77, 107, 122
4-Ethylgaiacol	122, 137, 153
Trichloroanisole	195, 210, 212
Geosmine	111, 112, 125
Tetrachloroanisole	231, 244, 246
2,4,6-Tribromoanisole	329, 344, 346
Pentachloroanisole	278, 280, 282

Table 3: SIM ions monitored for the target compounds

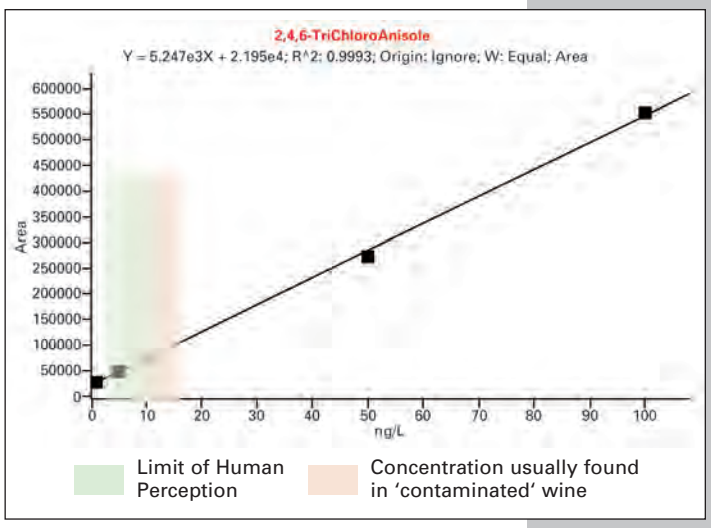

Figure 5: 2,4,6-Trichloroanisole from 2 to 5 ng/L

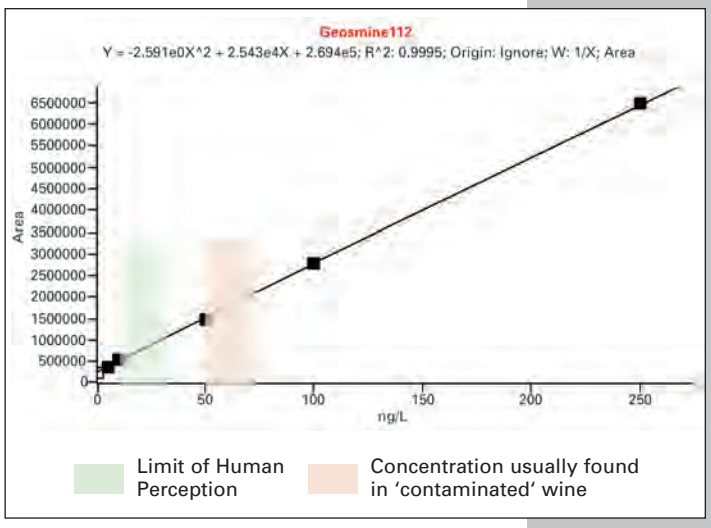

Figure 6: Geosmine from 10 to 50 ng/L

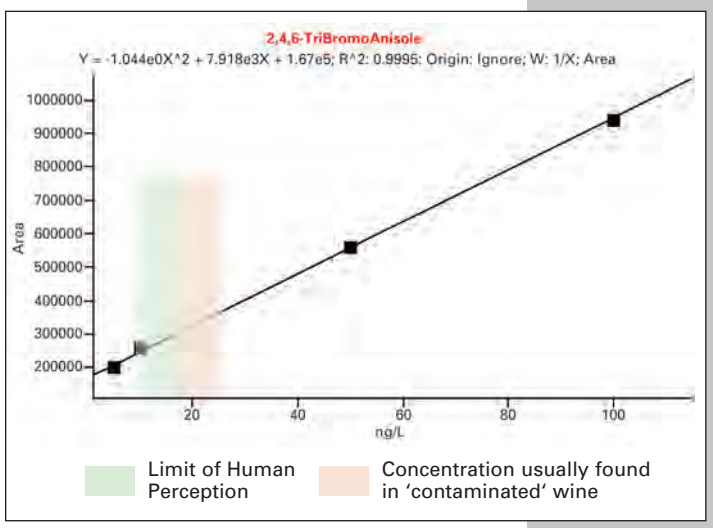

Figure 7: 2,4,6-Tribromoanisole from 10 to 20 ng/L

Conclusion

The ability of the ISQ GC-MS to detect several contaminants in wine at lower concentrations than the limit of human tasters, and its ease of use in combination with a single-step, two-minute sample preparation make it a useful tool for the wine industry. The sequential full-scan/SIM acquisition method for detecting the impurities also does not require extensive training of personnel to provide accurate results. In addition, this general method may be improved or customized to particular wines by incorporating new parameters such as trying other SPME coatings in the extraction phase.

The wine, champagne, and spirit market can be well served by analytical chemistry tools such as GC-MS. There are also other potential uses for this analysis method. For example, wine and other spirit producers risk their recipes being compromised when they outsource their product analysis, and prefer to conduct it on site. In addition, analysis of competitors' products using a GC-MS can help producers quantify what makes one wine superior to another.

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Determination of 1,4-Dioxane in Drinking Water by Gas Chromatography/Mass Spectrometry (GC/MS) with Selected Ion Monitoring (SIM)

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Application Note 52295

Key Words
ISQ Single Quadrupole GC-MS, TRACE GC Ultra, TriPlus RSH autosampler, PTV inlet, Sequential SIM/Full Scan, EPA Method 522, Environmental

Introduction
1,4-Dioxane is used mainly as a stabilizer for 1,1,1-trichloroethane for transport in aluminum containers. It is an irritant to eyes and respiratory system and suspected of causing damage to nervous system, liver, and kidneys.[1] In 2008, testing sponsored by the U.S. Organic Consumers Association found dioxane in almost half of tested organic personal-care products.[1] Of the total 1.163 million pounds of 1,4-dioxane released into the U.S. environment in 1992, as reported to the Toxics Release Inventory, 680 thousand pounds (58.5%) were released into the atmosphere, 450 thousand pounds (38.7%) were released into surface waters, and 33 hundred pounds (2.8%) were released onto the land (TRI92 1994).[2] In 2005, the New Hampshire Department of Environmental Services Waste Management Division started enforcement of an Ambient Groundwater Quality Standard reporting limit of 3 µg/L and trending towards a detection limit of 0.25 µg/L. 1,4-Dioxane has been detected in drinking water in the U.S. at a concentration of 1 µg/L. This application highlights the use of SIM/Full Scan to identify unknowns with a NIST library, while producing accurate results that meet EPA Method 522 requirements.

Experimental Conditions
Data was collected using a Thermo Scientific ISQ single quadrupole mass spectrometer utilizing the Thermo Scientific TriPlus RSH autosampler and a PTV inlet (CT-Splitless mode) on a Thermo Scientific TRACE GC Ultra gas chromatograph. The mass spectrometry data was collected in Full Scan (FS), selected ion monitoring (SIM), and SIM/Scan modes. A Thermo Scientific TraceGOLD TG-624 column (30 m × 0.25 mm ID, 1.4 µm film thickness; p/n 26085-3320) was used with a Siltek® deactivated baffle liner (p/n 453T2120). Table 1 lists the GC parameters. The ion source temperature of the mass spectrometer was set to 230 °C. The instrument was tuned to meet the bromofluorobenzene (BFB) criteria for this method. See Figure 1.

1,4-Dioxane calibration standards were prepared in dichloromethane as per the method to provide a range from 0.05 ppb to 40 ppb of dioxane.

Table 1. GC parameters

GC Oven Ramp		
Ramp	**Temp**	**Hold**
	30 °C	1 min
7 °C/min	90 °C	0 min
20 °C/min	200 °C	3 min
PTV Inlet		
Temperature	200 °C	
Split Flow	30 mL/min	
Splitless Time	0.50 min	
Solvent Valve Temp	100 °C	

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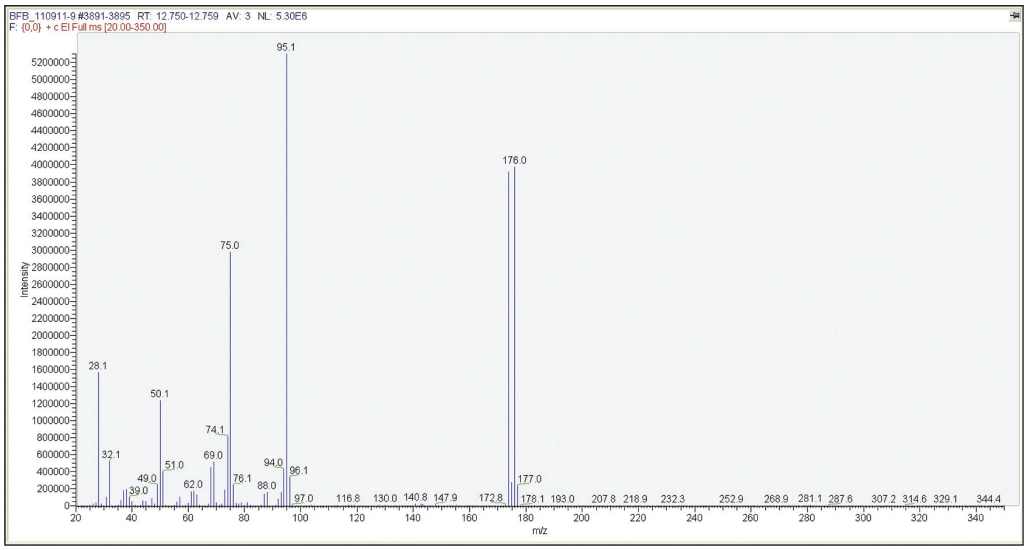

m/z	Criteria	Ion Intensity	TIC %	Criteria %	Pass/Fail
50	15%-40% of mass 95	871150	23.88	23.88	Pass
75	30%-80% of mass 95	1759792	48.25	48.25	Pass
95	Base peak	3647589	100.00	100.00	Pass
96	5%-9% of mass 95	240562	6.60	6.60	Pass
173	<2% of mass 174	21386	0.59	0.71	Pass
174	>50% of mass 174	2993264	82.06	82.06	Pass
175	5%-9% of mass 174	206831	5.67	6.91	Pass
176	>95% but <101% of mass 174	3003238	82.33	100.33	Pass
177	5%-9% of mass 176	173848	4.77	5.79	Pass

Figure 1. BFB and EPA Method 522 criteria

Full Scan Results

A calibration curve was created in Full Scan mode from 0.05 to 40 ppb of 1,4-dioxane. Figure 2 demonstrates the peak shape and S/N ratio at 0.1 ppb. The Full Scan calibration curve with an R^2 value of 0.9998 is presented in Figure 3.

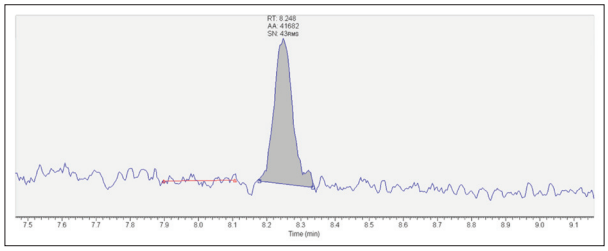

Figure 2. 1,4 -Dioxane at a concentration of 0.1 ppb with S/N = 43 in Full Scan

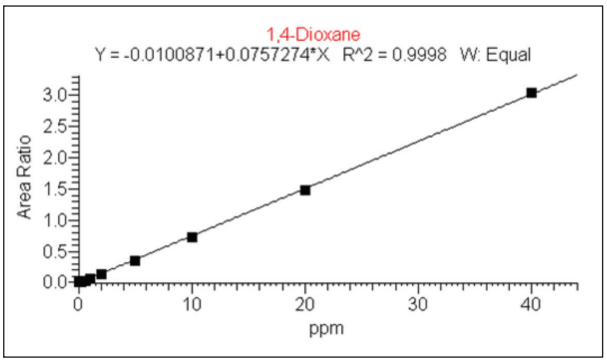

Figure 3. Full Scan calibration curve 0.05 to 40 ppb of 1,4-dioxane

SIM Results

A calibration curve was created in SIM mode from 0.05 to 40 ppb of 1,4-dioxane by monitoring three ions for the internal standard (46, 78, and 80), three ions for the surrogate (62, 64, 96), and two for the target compound (58, 88). Figure 4 shows the resulting calibration curve with an R^2 value of 0.9998. The chromatogram of the 0.05 ppb standard is depicted in Figure 5. At half the concentration of the full scan the S/N ratio is twice as high, highlighting the power of selected ion monitoring.

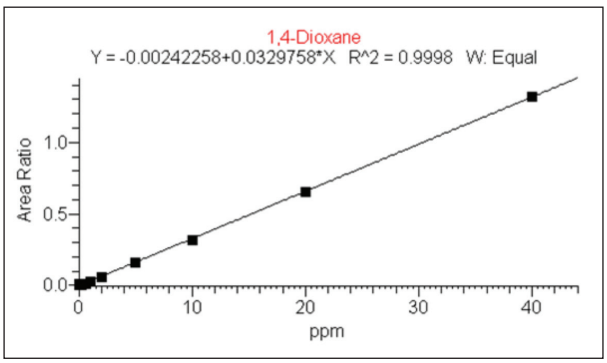

Figure 4. SIM mode calibration curve 0.05 to 40 ppb of 1,4-dioxane

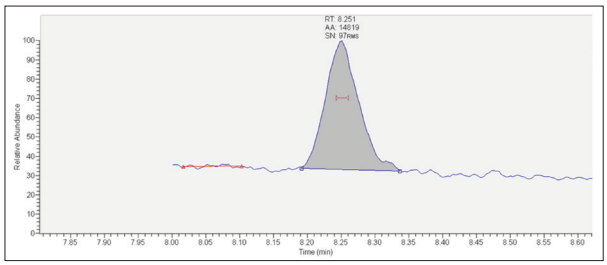

Figure 5. SIM analysis of 1,4-dioxane at 0.05 ppb with S/N = 97. Note the two-fold improvement in the S/N ratio in the SIM mode at one-half the concentration of 1,4-dioxane shown in the full scan in Figure 2.

Sequential SIM/Full Scan

The advantage of the SIM/Full Scan mode (tandem Full Scan/SIM) is the ability to identify additional peaks in unknown samples using a NIST or other library. Figure 6 provides the setup parameters for the SIM/Full Scan method in the software. Each scan segment contains both the SIM ions and scanning from 45 to 450 amu (Full Scan). SIM and the Full Scan alternate during the data collection. This is visualized in Figure 7, where the shorter scans are the SIM scans and the taller scans are the Full Scans. 1,4-Dioxane standards were analyzed from 0.05 to 40 ppb (Figure 8). According to EPA Method 522, each point on the curve must be within ± 20% of the true value, except the lowest point on the curve, which must be within ± 40%.[3] Even though the calibration curve is linear (R^2 = 0.9999), the curve only meets this criteria down to 0.5 ppb. By weighting the curve 1/x, the curve meets the criteria down to 0.05 ppb (Figure 9). Weighting the curve 1/x places more importance on the lower concentrations and has less influence in skewing the results, providing better accuracy at lower levels.

Time (min)	Mass List or Range (amu)	Dwell or Scan Times (sec)	Tune File Name
5.00	20-350	0.096	(Last Saved)
	46, 78, 80	0.03, 0.03, 0....	(Last Saved)
8.00	20-350	0.096	(Last Saved)
	58, 88, 62, 64, 96	0.03, 0.03, 0....	(Last Saved)

Figure 6. MS Method Parameters page from software showing SIM/Full Scan. Note that each segment can have its own specific tune file.

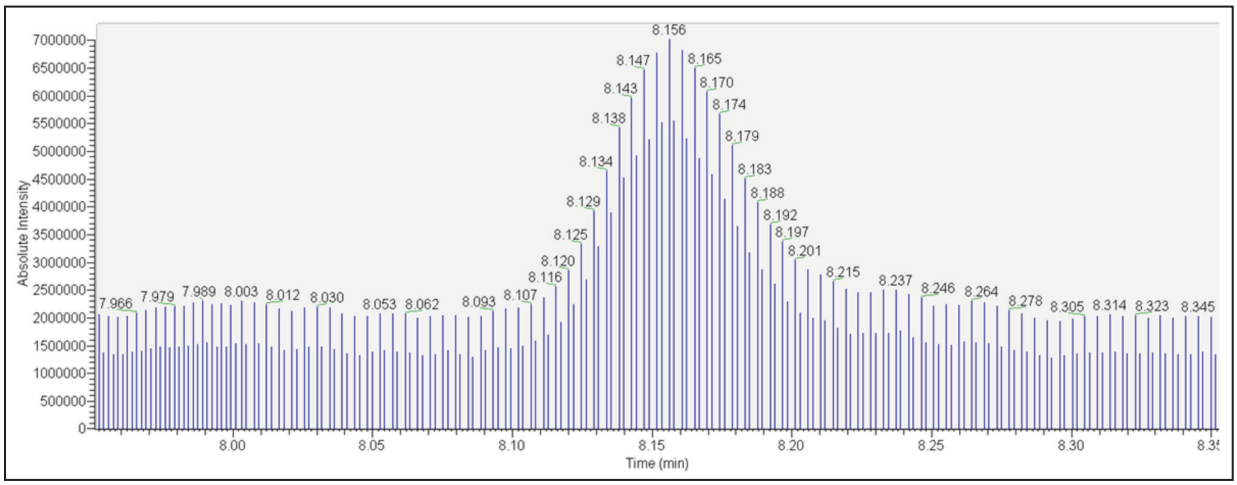

Figure 7. Chromatogram demonstrating the alternating SIM/Full Scan mode of data collection

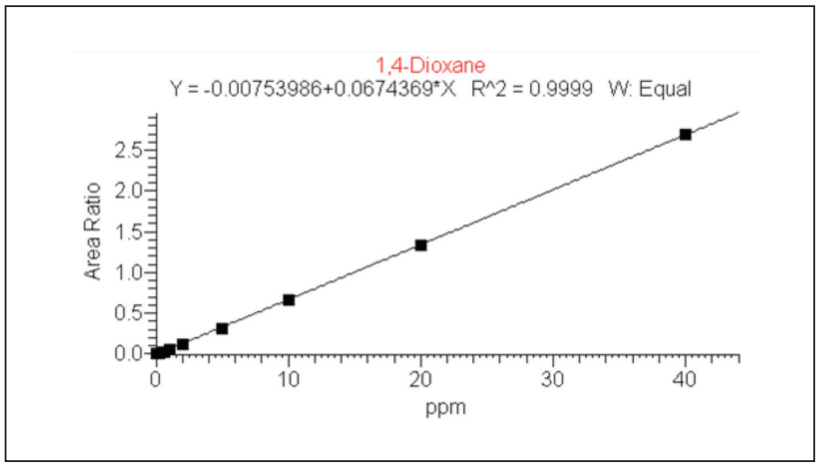

Figure 8. Sequential SIM/Full Scan calibration curve 0.05 to 40 ppb of dioxane

Specified Amount	Calculated Amount	Specified Amount	Calculated Amount
0.050	0.157	0.050	0.061
0.070	0.176	0.070	0.081
0.100	0.199	0.100	0.103
0.200	0.287	0.200	0.193
0.500	0.514	0.500	0.423
1.000	0.980	1.000	0.896
2.000	1.940	2.000	1.869
5.000	4.757	5.000	4.724
10.000	9.840	10.000	9.877
20.000	19.997	20.000	20.172
40.000	40.074	40.000	40.523

Figure 9. Equal weighting (left) vs. 1/x weighting (right) results for calibration curves. 1/x weighting provides better accuracy at lower concentrations

Comparison

Figure 10 is a comparison of the peak shape of 0.05 ppb in Full Scan, SIM and sequential SIM/Full Scan modes. No loss of precision or accuracy results from using SIM/Full Scan vs. SIM alone. However, by using the SIM/Full Scan mode additional compounds can be identified using a NIST or other library.

Reproducibility of the SIM/Full Scan mode was tested by injecting seven replicates from the same vial at concentrations of 0.07 and 2.0 ppb. The results are reported in Figure 11.

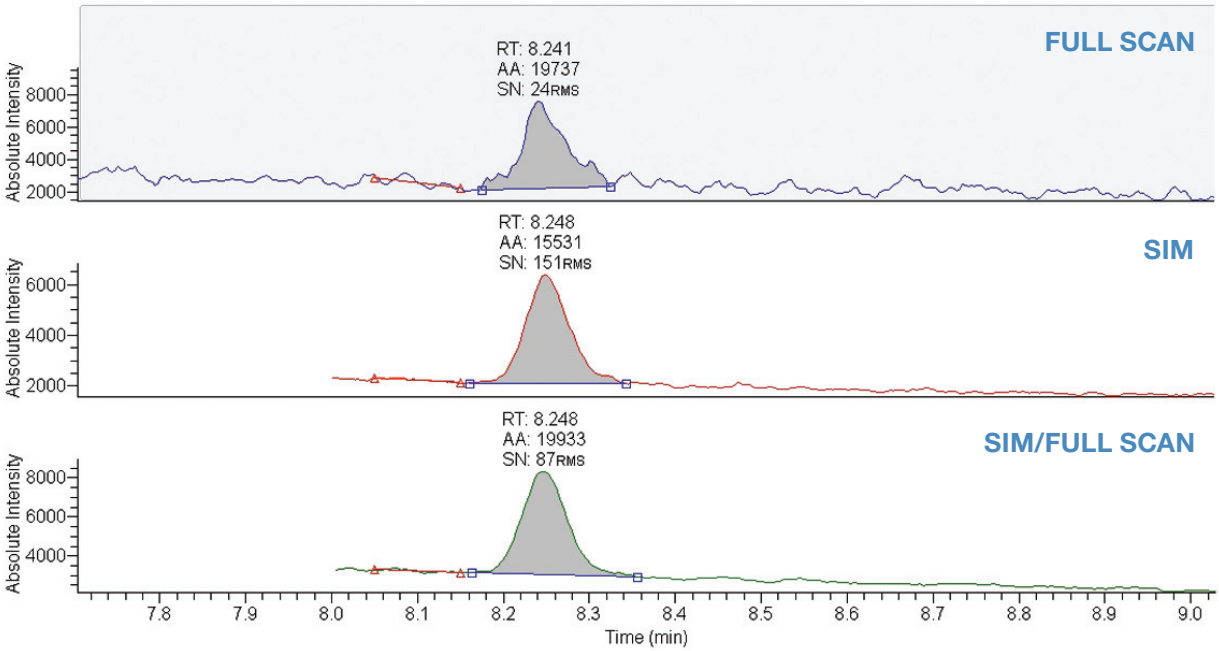

Figure 10. 0.05 ppb of 1,4-dioxane in Full Scan (S/N = 24), SIM (S/N = 151), and SIM/Full Scan (S/N = 87) modes

Sample Name	Area	ISTD Area	Area Ratio	Amount	RT
70ppt_Rep_2	28,335	14,060,852	0.002	0.069	8.236
70ppt_Rep_3	34,444	14,363,502	0.002	0.081	8.243
70ppt_Rep_4	31,241	13,625,849	0.002	0.078	8.234
70ppt_Rep_5	27,271	14,377,709	0.002	0.066	8.235
70ppt_Rep_6	31,189	14,662,503	0.002	0.073	8.234
70ppt_Rep_7	32,470	15,052,986	0.002	0.074	8.244
70ppt_Rep_8	38,823	15,153,194	0.003	0.086	8.240
Avg	*31,967*	*14,470,942*	*0.002*	*0.075*	*8.238*
StDev	*3,868*	*539,063*	*0.000*	*0.007*	*0.004*
%RSD	*12.10*	*3.73*	*10.33*	*9.13*	*0.05*

Sample Name	Area	ISTD Area	Area Ratio	Amount	RT
2ppm_Rep_2	823,612	15,064,599	0.055	1.655	8.238
2ppm_Rep_3	843,990	15,169,091	0.056	1.684	8.235
2ppm_Rep_4	857,227	15,163,169	0.057	1.711	8.231
2ppm_Rep_5	866,259	15,280,099	0.057	1.715	8.227
2ppm_Rep_6	822,302	14,467,495	0.057	1.720	8.239
2ppm_Rep_7	858,037	14,998,817	0.057	1.731	8.246
2ppm_Rep_8	839,242	14,638,036	0.057	1.735	8.236
Avg	*844,381*	*14,968,758*	*0.056*	*1.707*	*8.236*
StDev	*17,202*	*301,550*	*0.001*	*0.029*	*0.006*
%RSD	*2.04*	*2.01*	*1.68*	*1.67*	*0.07*

Figure 11. Precision in SIM/Full Scan mode at 0.07 and 2.0 ppb

Conclusion

The ISQ™ single quadrupole GC-MS system utilizing the TriPlus™ RSH autosampler and a PTV inlet (CT-Splitless mode) demonstrated its capability to analyze 1,4-dioxane according to EPA Method 522. It easily met the criteria for tuning with BFB and for calibration down to a level of 0.05 ppb. For better accuracy at the lower end of the curve, 1/x weighting was used to meet all of the criteria of the initial calibration of EPA Method 522. SIM analysis gave excellent results at low concentrations. The added advantage of the SIM/Full Scan mode is the ability to identify unknowns with a NIST or other library, while producing accurate results for 1,4-dioxane.

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4) EPA/600/R-08/101, Method 522 Determination of 1,4-Dioxane in Drinking Water by Solid Phase Extraction (SPE) and Gas Chromatography/Mass Spectrometry (GC/MS) with Selected Ion Monitoring (SIM), Version 1.0, September, **2008**, Jean W. Munch and Paul E. Grimmett.

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Method: 52147

A Solid-phase Micro-extraction GC-MS/MS Method for Rapid Quantitative Analysis of Food and Beverages for the Presence of Restricted Biologically Active Flavorings[1]

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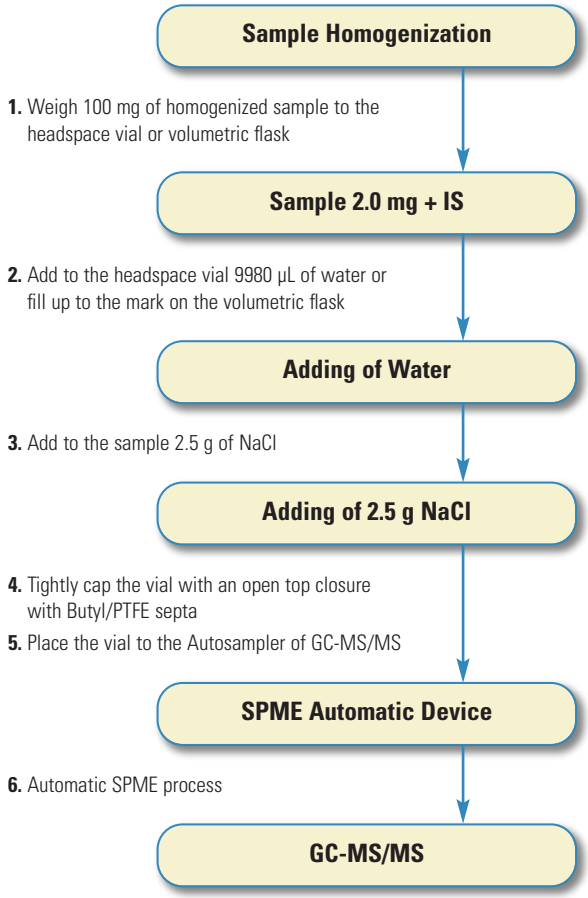
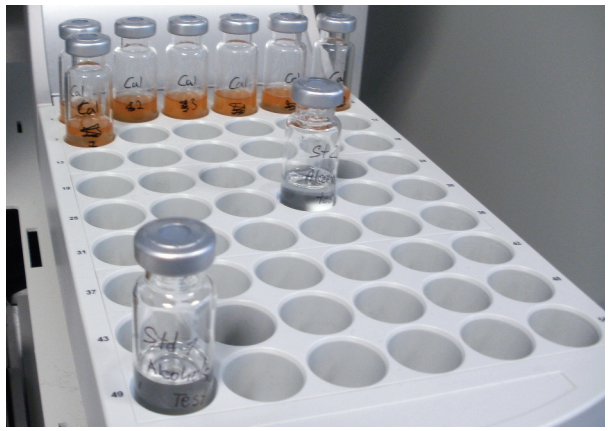
Key Words

- TSQ Quantum XLS
- Beverages
- Biologically Active Flavorings
- Semi-Solid Food
- Solid Food
- Solid-phase Micro-extraction

1. Schematic of Method





2. Introduction

Despite a history of use in foods and beverages, some plant extracts are now regulated in the EU[2] and some, such as safrole, are banned from direct addition to foods in the U.S.[3]

European Regulation 1334/2008[2] stipulates that 15 flavoring substances are banned from direct addition to foods or beverages in their chemically pure form. These flavoring compounds are agaric acid, aloin, capsaicin, coumarin, hypericine, β-asarone, estragole, hydrocyanic acid, menthofuran, methyleugenol, pulegone, quassin, safrole, teucrin A and α and β-thujone. Ten of these substances are permitted in food and beverages at stipulated levels, but only when they are naturally present in flavorings and food ingredients.[2] The stipulated foodstuffs cover a wide range of physical and chemical composition such as liquids (spirit drinks and non-alcoholic beverages), semi-solid foods (soups, sauces and desserts) as well as solid foods (confectionery, chewing gum, fish, meat, bakery products and breakfast cereals). Without methods that can be routinely applied by the food industry, it is very difficult to control final levels of these flavoring substances in finished products, especially due to their high variability in levels between different plant species.

Headspace analysis is a very attractive methodology for analyzing volatiles, because it requires minimal sample preparation and can be automated. Of the headspace methods, solid-phase micro-extraction (SPME) is now probably the most widely used method in food analysis, offering many benefits over other headspace techniques.[4] Although SPME is very well established for the analysis of flavorings, published methods have focused on individual food classes and no publications have truly tackled the issue of providing methodology for enforcement of regulations to control biologically active flavoring principles.

This publication describes a SPME method, utilizing a generic approach based on three categories of food types which has been optimized for the simultaneous determination of seven volatile flavoring substances whose levels are controlled in EU[2] in specified foods.

3. Scope

This method can be applied to alcoholic and non-alcoholic beverages, semi-solid processed foods and solid foodstuffs to detect and quantify the presence of seven biologically active flavoring substances (coumarin, β-asarone, estragole, menthofuran, methyl eugenol, pulegone and thujone) at levels ranging from 0.5 to 3000 mg/kg.

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4. Principle

The method employs automated headspace solid-phase micro-extraction (HS/SPME) for extraction of the targeted compounds (biologically active flavorings) from very broad types of matrices using a polydimethylsiloxane (PDMS) SPME fiber. Samples are placed in the headspace vials, fortified with labeled internal standards and water along with sodium chloride (NaCl) is added. Headspace vials are tightly sealed and after achieving equilibration headspace partition, the headspace is sampled automatically and analyzed by simultaneous GC-MS/MS using a Thermo Scientific TSQ Quantum XLS gas chromatography triple quadrupole mass spectrometer system.

5. Reagent List

		Fisher Scientific Part Number
5.1	Purified Water (obtained from Thermo Scientific Barnstead EASYpure II water system)	3125753
5.2	Sodium Chloride (extra pure)	194090010
5.3	Saccharose (extra pure)	S/8560/53
5.4	Ethanol (purity 99.9%)	E/0665DF/17
5.5	Methanol (purity 99.9%)	M/4058/17

6. Calibration Standards

6.1 Biologically Active Flavorings

6.1.1 Beta-asarone – purity 72% (Dr. Ehrenstorfer)

6.1.2 Coumarin (1,2-benzopyrone) – purity 99.5% (Dr. Ehrenstorfer)

6.1.3 Estragole (1-Allyl-4-methoxybenzene) – purity ≥ 98.5% (Sigma-Aldrich)

6.1.4 Menthofuran – purity ≥ 99% (Sigma-Aldrich)

6.1.5 Methyleugenol (4-Allyl-1,2-dimethoxybenzene) – purity 99.5% (Sigma-Aldrich)

6.1.6 Pulegone – purity 98.8% (Sigma-Aldrich)

6.1.7 Thujone (alpha and beta) – purity ≥ 99% (Sigma-Aldrich)

6.2 Internal Standards

6.2.1 Coumarin – 5, 6, 7, 8 – D4, c = 100 µg/mL in acetone (Dr. Ehrenstorfer)

6.2.2 Dicyclohexylmethanol – purity 98% (Sigma-Aldrich)

7. Standards Preparation

7.1 Stock standard solutions of flavorings (1000 µg/mL): Weigh 25.00 mg of the compound (recalculate the amount regarding actual purity of the standard) into volumetric flasks, dissolve in methanol and dilute to 25 mL. Solutions can be stored at 4 °C for at least three months.

7.2 Working standard solution of 7 flavorings (1 respectively 10 µg/mL for coumarin): Transfer 25 µL of stock solution of thujone, menthofuran, estragole, pulegone, methyl eugenol and β-asarone (1000 µg/mL) and 250 µL of stock solution of coumarin (1000 µg/mL) to a 25 mL volumetric flask and dilute to marked volume with water. Solution should be prepared fresh every time before using.

7.3 Stock standard solution of internal standard dicyclohexylmethanol (1000 µg/mL): Weigh 25.00 mg of the compound (recalculate the amount regarding actual purity of the standard) into a volumetric flask, dissolve in methanol and dilute to 25 mL. Solution can be stored at 4 °C for at least three months.

7.4 Working standard solution of internal standard dicyclohexylmethanol (10 µg/mL): Transfer 100 µL of stock solution of dicyclohexylmethanol (1000 µg/mL) to a 10 mL volumetric flask and dilute to marked volume with water. Solution should be prepared fresh every time before using.

8. Apparatus

		Fisher Scientific Part Number
8.1	High speed blender – ULTRA-TURRAX®	3565000
8.2	ULTRA-TURRAX – Dispergation tool	1713300
8.3	ULTRA-TURRAX – Plug-in coupling	1024200
8.4	Waring laboratory blender	68909
8.5	Fisher precision balance	XP-1500FR
8.6	Sartorius analytical balance	ME235S
8.7	SPME holder – TriPlus™ SPME Kit	190.504.34

8.8 TSQ Quantum XLS™ Triple Quadrupole Mass Spectrometer – Thermo Fisher Scientific (Austin, TX USA)

8.9 Thermo Scientific TRACE GC Ultra system with automated SPME system – Thermo Fisher Scientific (Austin, TX USA)

9. Consumables

		Fisher Scientific Part Number
9.1	GC column – TraceGOLD TG-5MS 5% diphenyl and 95% dimethyl polysiloxane stationary phase, 30 m, 0.25 mm ID, 0.25 µm film thickness (Thermo Fisher Scientific, Bellefonte, PA USA)	26098-1420
9.2	SPME fiber – coated with PDMS, d_f 100 µm	57341-U *Supelco Bellefonte, PA USA*
9.3	Headspace vials – 20 mL flat bottom, clear glass, beveled edge	3205551
9.4	PTFE – faced butyl rubber septa for headspace vials – 20 mm, septa Butyl/PTFE	3205532
9.5	Capping device – Manual Crimper for 20 mm Aluminum Crimp Seals	C4020-100
9.6	Pipette Finnpipette 100–1000 µL	3214535
9.7	Pipette Finnpipette 20–200 µL	3214534
9.8	Pipette Finnpipette 10–100 µL	3166472
9.9	Pipette Finnpipette 500–5000 µL	3166473
9.10	Pipette Finnpipette 1000–10000 µL	3214536
9.11	Pipette holder	3651211
9.12	Pipette tips 0.5–250 µL, 500/box	3270399
9.13	Pipette tips 1–5 mL, 75/box	3270420
9.14	Pipette tips 100–1000 µL, 200/box	3270410
9.15	Pipette tips 20000–10000 µL, 40/box	3270425
9.16	Pipette Pasteur – soda lime glass 150 mm	FB50251
9.17	Pipette suction device	3120891
9.18	Spatula, 18/10 steel	3458179F
9.19	Spatula, nylon	3047217
9.20	Wash bottle, PTFE	3149330
Glassware		
9.21	Beaker, 50 mL	965 32 10
9.22	Beaker, 100 mL	965 32 20
9.23	Volumetric flask, 10 mL	FB50143
9.24	Volumetric flask, 25 mL	FB50147

10. Procedure

Preparation of the Instrument – Before starting to work with the instrument or preparation it for work in SPME mode, please read carefully the relevant chapter of the Thermo Scientific TriPlus Operating Manual and Section IV in the Thermo Scientific TriPlus Standard Operating Procedures. There is described all necessary maintenance during installation of the SPME holder and SPME fiber.

10.1 Sample Preparation

Solid and Semi-solid matrices

10.1.1 Homogenize 150 g of sample in a high-speed blender (soups, sauces and pesto) or in a Waring laboratory blender (solid matrices like muesli) for 5 min, and then accurately weigh 0.1 g directly into a headspace vial.

10.1.2 Add 10 µL of working standard solution of dicyclohexylmethanol, 10 µL of standard solution of coumarin-d_4, and add 9980 µL of water using micropipettes of appropriate sizes.

Liquid Matrix

10.1.3 Weigh 0.1 g directly into a headspace vial add 10 µL of working standard solution of dicyclo-hexylmethanol, 10 µL of standard solution of coumarin-d_4 and add 9980 µL of water using micropipettes of appropriate sizes.

10.1.4 In both cases, add 2.5 g NaCl, seal with a PTFE-faced butyl rubber septum and cap the sample with the crimping device.

10.1.5 For calibration purposes, use blank foodstuffs representative of each of the respective matrix types.

- Liquid matrix (mainly representing alcoholic drinks) comprising a 40% solution of aqueous ethanol used as blank material
- Semi-solid matrix (mainly representing sauces and pesto) comprising pure tomato sauce used as blank material
- Solid matrix (mainly representing muesli) comprising oat flakes used as blank material

For solid and semi-solid matrices, use the volumes of standards and internal standards as shown in Table 1, and for liquid matrices use corresponding volumes as shown in Table 2.

10.2 Automated SPME Analysis

10.2.1 Use the fiber coated with polydimethylsiloxane 100 µm (PDMS-100) and condition the fiber before use by insertion into the GC injector as recommended by the manufacturer.

10.2.2 Load the SPME autosampler with headspace vials containing the prepared samples (up to a maximum of 54 vials per tray).

10.2.3 Commence the SPME program which consists of swirling the vial for 5 min at 50 °C, then inserting the fiber into the head-space for 40 min at 50 °C as the solution is swirled again, then transferring the fiber to the injector for desorption at 250 °C for 5 min. At the end of the program, the fiber is transferred to the second injector (instead of the conditioning station) for cleaning and conditioning at 250 °C for 5 min.

10.3 GC Analysis

GC analysis is performed on a TRACE GC Ultra™ system with automated SPME system (Thermo Fisher Scientific, Austin, TX USA). The GC conditions were as follows:

Column: TraceGOLD TG-5MS 5% diphenyl and 95% dimethyl polysiloxane stationary phase (30 m, 0.25 mm ID, 0.25 µm film thickness)

Injection mode: splitless

Injection port temperature: 250 °C

Left carrier flow: 1.2 mL/min

Split flow: 50 mL/min

Splitless time: 3 min

Conditioning injector temperature: 250 °C

Right carrier flow: 0.1 mL/min

Transfer line temperature: 250 °C

Oven temperature: 60 °C hold for 1 min; to 120 °C with 15 °C/min; hold for 2 min; to 225 °C with 30 °C/min; hold for 1 min; to 280 °C at 30 °C/min, hold for 10 min

10.4 Tandem MS/MS Detection

MS analysis is carried out using a TSQ Quantum XLS triple quadrupole mass spectrometer (Thermo Fisher Scientific, Austin, TX USA).

Ionization mode: electron impact (EI) positive ion at 70 eV ionization energy

Emission current: 30 µA

Ion source temperature: 250 °C

Scan type: selected reaction monitoring (SRM)

Cycle time: 0.1 s

Peak width: Q1/Q3 the full width of a peak at half its maximum height (FWHM) of 0.70 Da

Collision gas (Ar) pressure: 1.0 mTorr

The parameters for selected reaction monitoring (SRM) analysis for targeted compounds and internal standards are displayed in the Table 3.

11. Calculations of Results

11.1 Identification

It is confirmed by the presence of transition ions (quantifier and qualifier) at retention times (±0.05%) to the corresponding standards. In multiple reaction monitoring (MRM) mode the measured peak area ratios for qualifier to quantifier ion should be in close agreement (±20%) with those of the standards as shown in Table 3. The quantifier and qualifier ion were selected among the product ions produced by the fragmentation of the selected parent ion on the basis of the intensity.

11.2 Quantification

It employs internal standardization using peak area ratios for standards in matched matrices. Dicyclohexylmethanol is used as the internal standard for the six flavor compounds (thujone, menthofuran, estragole, pulegone, methyl eugenol and β-asarone), and coumarin-d_4 is used as internal standard for coumarin. Plot the calibration curves as the relative peak areas (analyte versus the corresponding internal standard) as a function of the compound concentration. The flavoring concentration (c_f) in the samples is determined from the equation:

$$c_{Fl} = \left(\frac{A_{Fl}}{A_{IS}}\right) - b/a$$

where,

c_{Fl} – flavoring concentration in mg/kg

A_{Fl} – peak area of the flavoring

A_{IS} – peak area of internal standard

b – the y-intercept

a – the slope of calibration curve

Samples initially found to contain levels of flavoring substances outside the linear range need to be appropriately diluted, and the dilution factor taken into account in the final calculations.

12. Method Validation

Validation was carried out in terms of specificity, linearity, precision, limit of detection (LOD) and quantification (LOQ), accuracy and robustness. Finally, the applicability of the method to the determination of targeted flavorings in a number of commercial samples was demonstrated.

The method performance was established by spiking experiments with blank matrices (solid – oat flakes; semi-solid – pure tomato sauce; and liquid – water with ethanol) with a mixture of targeted compounds.

12.1 Specificity

Using Selected Reaction Monitoring (SRM) the specificity is confirmed based on the presence of the transition ions (quantifier and qualifier) at the correct retention times corresponding to those of the respective flavoring standards. The measured peak area ratios of qualifier/quantifier ion have to be in close accordance with the ion ratios of the standards as indicated in Table 3.

12.2 Linearity and Calibration Curve

The linearity of calibration curves is assessed over the range from 0.01–2.0 mg/kg (for six flavorings) and 0.1–2.0 mg/kg for coumarin. In all cases, the correlation coefficients of linear functions has to be > 0.99. The calibration curves are created from seven matrix-matched calibration standards which are injected in each batch in duplicate.

12.3 Precision

The relative standard deviation (% RSD) was determined by injecting six replicates of spiked samples of three different matrices at two different levels. For the liquid matrix, aqueous ethanol (40%) was used as the blank matrix (with the addition of various amounts of saccharose to simulate liqueurs and energy drinks), for semi-solid matrices pure tomato sauce was used, and for the solid matrix oat flakes. The samples were spiked at 0.1 and 1 mg/kg levels and six replicate analyses were analyzed. For six flavorings (β-asarone, estragole, menthofuran, methyl eugenol, pulegone and thujone) the first level of addition was 0.1 mg/kg, and for coumarin the addition was at 1 mg/kg. The second level of addition for six flavorings was 1 mg/kg, again with coumarin being at a higher level of 10 mg/kg. The results that establish method precision are shown in Table 4, indicating RSDs from 2 to 21%. All precisions are acceptable for a regulatory method, with liquid and semi-solid foods offering a better performance than solid foods.

12.4 Limits of Detection (LOD) and Quantification (LOQ)

Limits of detection and quantification were estimated following the IUPAC approach which consisted of analyzing the blank sample to establish noise levels and then estimating LODs and LOQs for signal/noise, 3 and 10 respectively. The values for three matrices (solid, semi-solid and liquid) are shown in Table 5 and, in all cases, these values far exceed requirements to test for compliance to regulatory limits in which 0.5 mg/kg is the lowest level which is controlled.

12.5 Accuracy

Accuracy was evaluated by comparing found values with spikes by standard addition. The optimization method was used to analyze three types of matrix. For the liquid matrix, spiking was into 40% aqueous ethanol, for a semi-solid matrix pure tomato sauce was used, and for a solid matrix oat flakes were used. The samples were spiked at levels of 0.1 and 1 mg/kg in six replicates. For six flavorings (β-asarone, estragole, menthofuran, methyl eugenol, pulegone and thujone) at 0.1 mg/kg and for coumarin at 1 mg/kg for level 1, and six flavorings at 1 mg/kg and 10 mg/kg for coumarin for level 2. The results in Table 6 show good accuracy, except in the case of solid matrices for which overestimations are indicated.

13. Conclusion

This single laboratory validated method is capable of determining levels of any one of seven biologically active flavoring substances which have use restrictions in composite foodstuffs. The method can cover all food types based on a generic approach of selecting the category of either a liquid, semi-solid or solid matrix, and then following the optimized conditions for that category. The method has a sensitivity which far exceeds regulatory requirements and the use of MS/MS for detection guarantees a high level of confidence in correct identification based on ion ratios. We recommend this method for use for enforcement of limits of biologically active flavorings in foods.

14. References

1. For details of this research please see: Bousova K., Mittendorf K., Paez V., Senyuva H., A Solid-Phase Micro-Extraction GC-MS/MS Method for Rapid Quantitative Analysis of Food and Beverages for the Presence of Legally Restricted Biologically Active Flavorings. *J. AOAC.* Accepted for publication, 2011.
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Concentrations of 6 Flavoring Standards + Coumarin (mg/kg)	Concentration of DCHM & coumarin-d_4 Standard (mg/kg)	Volume of Working Standard Solution Added (µL)	Volume of Working Internal Standard DCHM Added (µL)	Volume of Standard Solution of coumarin-d_4 Added (µL)	Volume of Water Added (µL)
0.01 and 0.1	1 and 10	1	10	10	9979
0.05 and 0.5	1 and 10	5	10	10	9975
0.1 and 1	1 and 10	10	10	10	9970
0.5 and 5	1 and 10	50	10	10	9930
1 and 10	1 and 10	100	10	10	9880
1.5 and 15	1 and 10	150	10	10	9830
2 and 20	1 and 10	200	10	10	9780

DCHM = dicyclohexylmethanol internal standard

Table 1: Preparation of matrix matched standards for semi-solid and solid matrices

Concentrations of 6 Flavoring Standards and Coumarin (mg/kg)	Concentration of DCHM & coumarin-d_4 Standard (mg/kg)	Volume of Working Standard Solution Added (µL)	Volume of Working Internal Standard DCHM Added (µL)	Volume of Standard Solution of coumarin-d_4 Added (µL)
0.01 and 0.1	1 and 10	1	10	10
0.05 and 0.5	1 and 10	5	10	10
0.1 and 1	1 and 10	10	10	10
0.5 and 5	1 and 10	50	10	10
1 and 10	1 and 10	100	10	10
1.5 and 15	1 and 10	150	10	10
2 and 20	1 and 10	200	10	10

DCHM = dicyclohexylmethanol internal standard

Table 2: Preparation of matrix matched standards for liquid samples

Flavoring Substance	Retention Time (min)	Molecular Weight	Precursor Ion	Quantifier Ion	Qualifier Ion 1	Ion Ratio Qual/Quant	Collision Energy (V)
Thujone	5.86	152.23	110.03	95.02	67.05	0.20	10
Menthofuran	6.66	150.22	107.94	79.01	77.00	0.52	15
Estragole	7.16	148.20	147.98	91.06	115.10	0.82	25
Pulegone	7.74	152.23	152.01	81.03	137.04	0.44	10
Methyl eugenol	9.18	178.23	177.98	147.03	163.05	0.72	15
Coumarin	9.50	146.14	145.92	117.99	89.93	0.68	20
coumarin-d_4	9.49	150.17	149.92	122.02	93.98	0.14	15
Dicyclohexylmethanol	10.42	196.33	112.27	79.05	81.05	0.90	10
β-Asarone	10.43	208.26	207.99	165.08	193.11	0.91	15

Table 3: GC-MS/MS parameters for selected reaction monitoring of flavorings

Analyte	Relative Standard Deviation (RSD %)							
	Level 1 mg/kg	Level 2 mg/kg	Liquid Matrix		Semi-solid Matrix		Solid Matrix	
			Level 1	Level 2	Level 1	Level 2	Level 1	Level 2
Thujone	0.1	1	6	5	8	5	17	17
Menthofuran	0.1	1	14	5	2	15	19	21
Estragole	0.1	1	6	4	9	8	17	13
Pulegone	0.1	1	3	6	7	5	8	16
Methyl eugenol	0.1	1	7	6	3	4	9	12
Coumarin	1	10	13	2	13	3	13	7
β-Asarone	0.1	1	8	11	6	5	4	10

Table 4: RSD (%) of 6 spiked samples at 2 levels

Analyte	Liquid Matrix		Semi-solid Matrix		Solid Matrix	
	LOD (mg/kg)	LOD (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)	LOQ (mg/kg)	LOQ (mg/kg)
Thujone	0.001	0.005	0.01	0.05	0.003	0.01
Menthofuran	0.001	0.005	0.003	0.01	0.005	0.01
Estragole	0.005	0.01	0.001	0.005	0.005	0.01
Pulegone	0.001	0.005	0.003	0.01	0.001	0.005
Methyleugenol	0.001	0.005	0.01	0.05	0.01	0.05
Coumarin	0.1	0.5	0.1	0.5	0.1	0.5
β-Asarone	0.001	0.005	0.01	0.05	0.003	0.01

Table 5: Limits of detection and quantification (LODs and LOQs)

Analyte	Recoveries (%)							
	Level 1 mg/kg	Level 2 mg/kg	Liquid Matrix		Semi-solid Matrix		Solid Matrix	
			Level 1	Level 2	Level 1	Level 2	Level 1	Level 2
Thujone	0.1	1.0	95	99	83	121	146	131
Menthofuran	0.1	1.0	121	83	50	83	126	124
Estragole	0.1	1.0	115	90	129	125	123	117
Pulegone	0.1	1.0	107	88	98	105	119	127
Methyl eugenol	0.1	1.0	99	91	106	102	124	113
Coumarin	1.0	10.0	96	97	96	111	107	111
β-Asarone	0.1	1.0	85	121	62	90	115	116

Table 6: Recoveries (%) for spiked samples at 2 levels

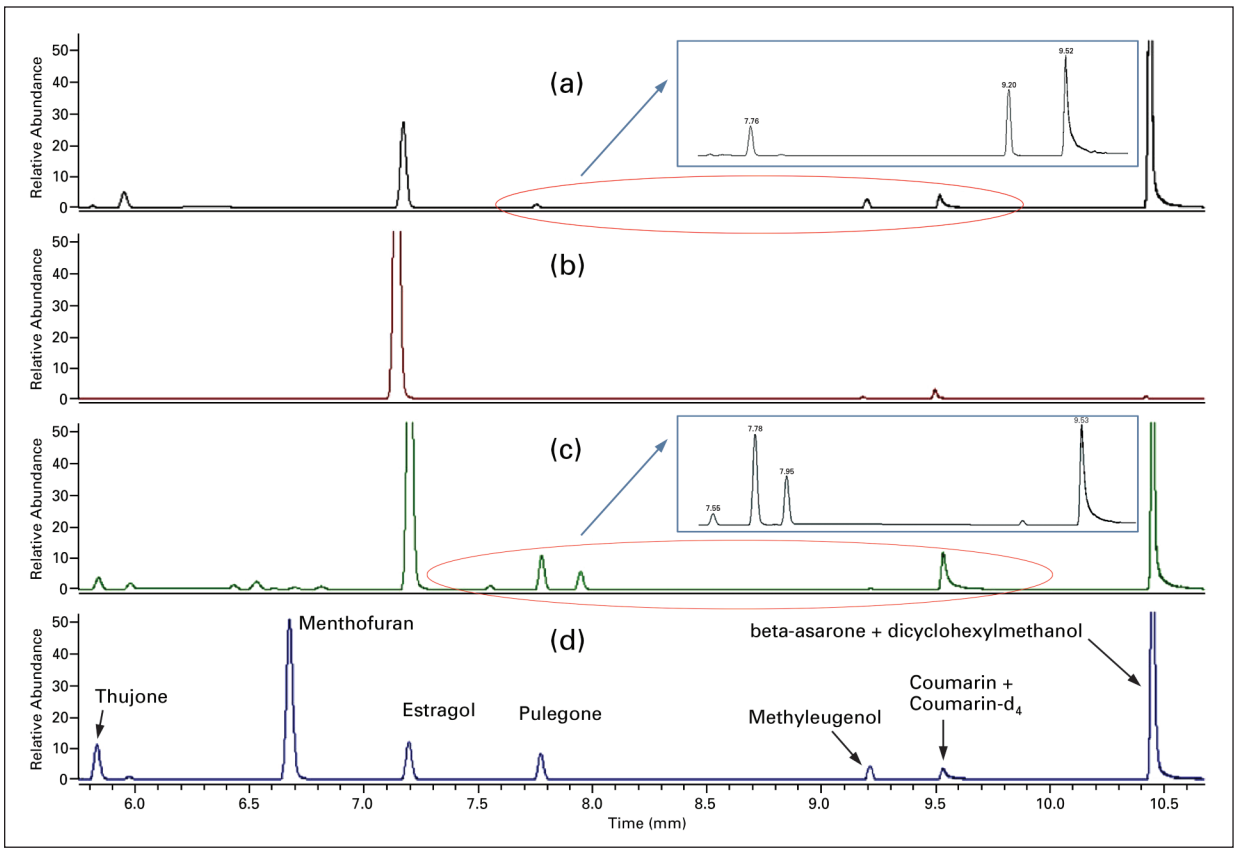

Figure 1: MRM chromatograms for (a) Herbal liqueur containing estragole, pulegone & methyl eugenol; (b) Pesto sauce containing estragole and methyl eugenol; (c) Herbal tea containing thujone, menthofuran, estragole, pulegone, methyl eugenol and coumarin; (d) mixture of seven flavoring standards and two internal standards

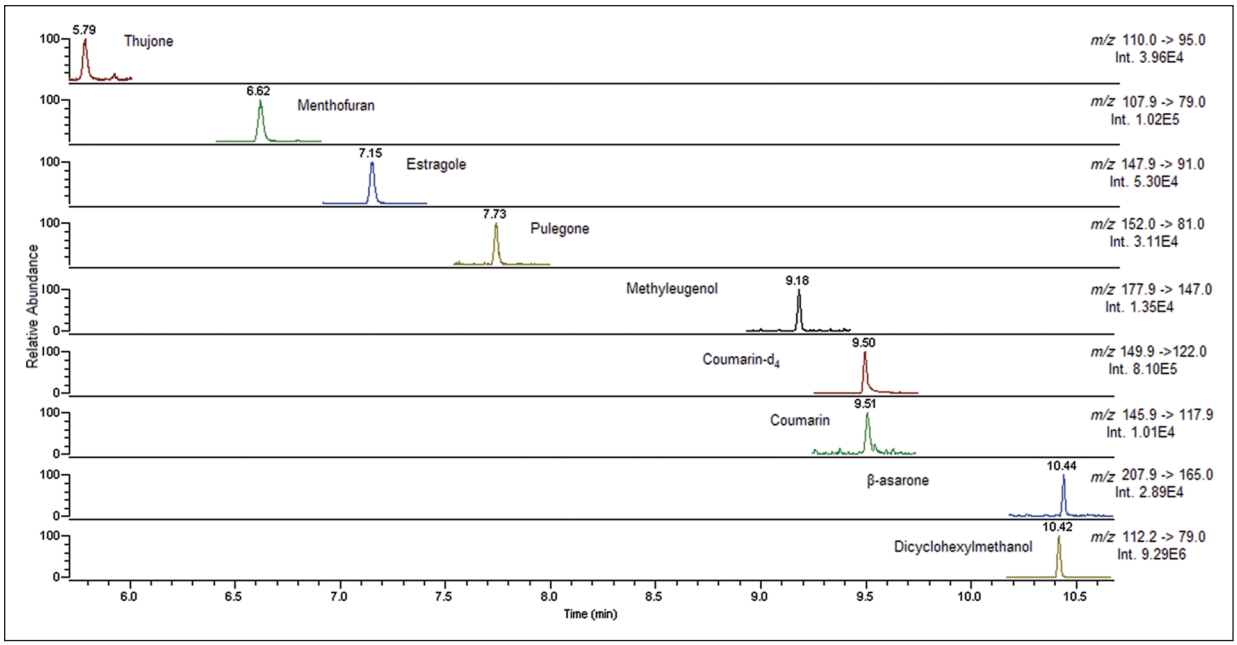

Figure 2: Chromatogram of the matrix matched standard for semi-solid matrixes (pure tomato sauce used as blank material) with concentration 0.01 mg/kg for β-asarone, estragole, menthofuran, methyleugenol, pulegone and thujone; 0.1 mg/kg for coumarin and for internal standards 1 mg/kg for dicyclohexylmethanol and 10 mg/kg for coumarin-d_4. The figure shows SRM traces for 7 flavoring substances plus 2 internal standards.

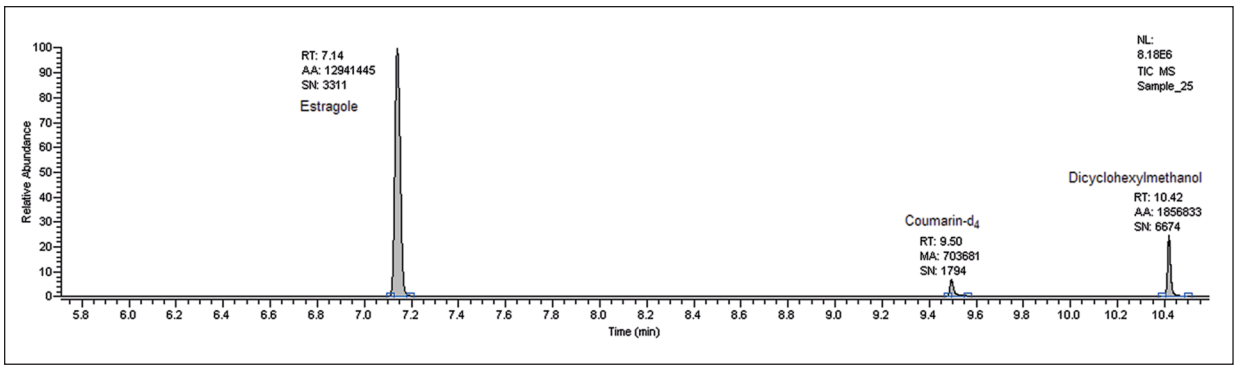

Figure 3: Chromatogram of basil tomato sauce; detected flavoring substance: estragole – 3.54 mg/kg; internal standards: coumarin-d_4 – 10 mg/kg and dicyclohexylmethanol – 1 mg/kg

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Application Notebook

About GC and GC-MS Technology

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Thermo Scientific TSQ 8000 GC-MS/MS Software Overview

Introduction

The advantages of selected reaction monitoring (SRM) and multiple reaction monitoring (MRM) analysis are often accompanied by some challenges, especially with respect to the adoption in the laboratory. These challenges can be due to the complexity of the mass spectrometer setup and optimization. Therefore, laboratories wanting to realize the benefits of MS/MS must overcome some barriers in the tuning, setup and optimization to reduce the lead time to get into a routine workflow.

The Thermo Scientific TSQ 8000 GC-MS/MS was built with simplicity as a priority. Whether you are managing retention times, starting with a completely new analysis, transferring a method from a single quadrupole GC-MS to a triple quadrupole GC-MS, or porting a known MRM method from another instrument, the TSQ™ 8000 GC-MS/MS system, through its integrated software tools, ensures the fastest route to high performance results routinely.

The TSQ 8000 GC-MS/MS facilitates your lab day:

- maintains actual compound retention times
- imports the last found retention time - for example, from QC samples - into data acquisition and/or data processing software
- updates shifting retention times with matrix samples
- compensates for aging columns
- provides an easy update of the retention time after column clipping
- locks retention time automatically for all compounds in the acquisition list
- provides safe compound identification based on retention time or retention index

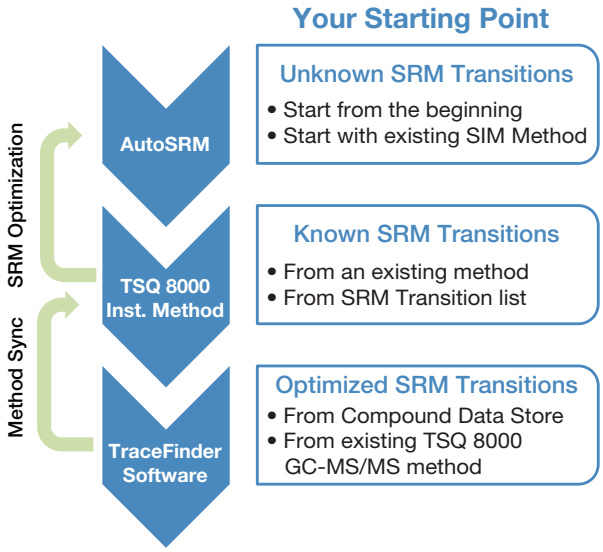

Figure 1. Workflow diagram of TSQ 8000 GC-MS/MS software. The design goal of the software was to make it flexible and easy to use, regardless of what your starting point is in developing your SRM method.

Software Components

Figure 1 shows a workflow diagram of the three software components of the TSQ 8000 GC-MS/MS system:

- **AutoSRM Capability:**
  Purpose-built software for SRM creation and optimization
- **TSQ 8000 GC-MS/MS Instrument Method:**
  Offers true timed SRM operation, allowing for high sensitivity and ease of use for the most complex SRM methods
- **Thermo Scientific TraceFinder Software:**
  A multi-platform user-friendly chromatography analysis software

The sections that follow describe how these components interact to quickly get you from your starting point to the routine analysis of your samples.

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Starting from Scratch or from a SIM Method

AutoSRM assists and automates much of the process of creating SRM transitions from the beginning. If you already have a SIM method as a starting point, the process is even faster. Figure 2 shows a collision energy optimization curve for 1, 2-trichlorobenzene obtained using AutoSRM. From here, simply export your optimized transition list to the instrument method, and you are ready to run. For more details on how AutoSRM assists in SRM development see *Application Brief AB52298: Introducing AutoSRM.*

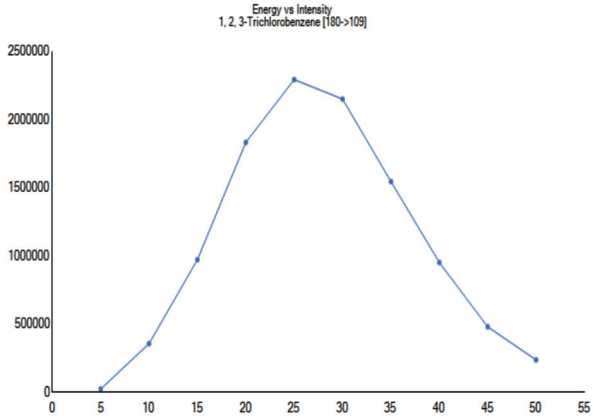

Figure 2. SRM collision energy optimization curve obtained using AutoSRM for 1, 2, 3 tricholobenzene.

Starting from an SRM Method

If you already have an SRM method, you can import your transition list into a TSQ 8000 instrument method either from a supported instrument method type or from a spreadsheet with your transition list. The transition list can then be exported to TraceFinder™ software for processing setup or to AutoSRM for further optimization. Figure 3 shows a timed acquisition list in the TSQ 8000 instrument method. For details on the instrument method, see *Application Brief AB52299: The TSQ 8000 Triple Quadrupole GC-MS/MS Instrument Method.*

Starting from Compound Data Store

If you already have a database of SRMs in TraceFinder software, simply choose your compound list, as shown in Figure 4. That's it! TraceFinder software will create your acquisition list and processing method from your selection.

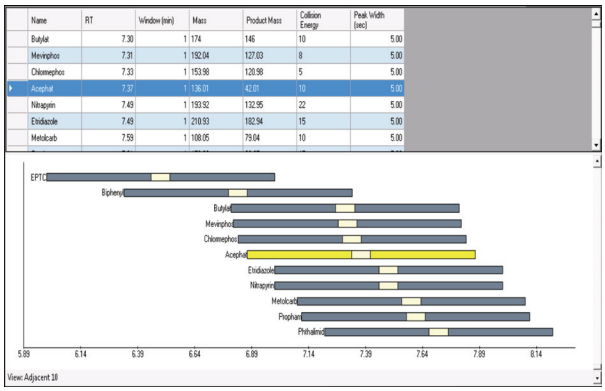

Figure 3. Timed-SRM acquisition list as seen in the TSQ 8000 instrument method. If you already know your SRM transitions, you can import the list using common industry method formats or from a spreadsheet. The compound name is listed on the left side of the bar. The white section in the middle of the bar indicates the expected peak width. The left portion of the bar indicates the acquisition time before peak elution, and the right portion indicates the acquisition time after peak elution. Flexible settings for tailing peaks are possible.

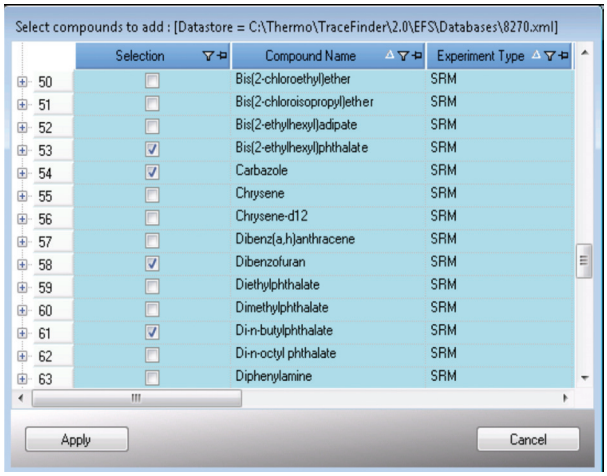

Figure 4. Building a method using TraceFinder software. Simply select the compounds to acquire and both acquisition list and processing methods are created for you.

Making Retention Time Adjustments

To make minor retention time adjustments, run a standard and you are ready to run samples. Retention time adjustments that are made in data review are pushed back to your timed acquisition method, simplifying method maintenance. For more details on how the TSQ 8000 instrument method can be synced to TraceFinder software, see *Application Brief AB52300: TSQ 8000 GC-MS/MS Method Sync.*

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Introducing AutoSRM

MRM Simplicity for High Performance Results

Overview

The advantages of selected reaction monitoring (SRM) and multiple reaction monitoring (MRM) analyses are often accompanied by some challenges, especially with respect to the adoption of these methods in the laboratory. These challenges can be due to the complexity of the mass spectrometer setup and optimization. Laboratories that want to realize the benefits of MS/MS must overcome some barriers in the tuning, setup and optimization to reduce the lead time to get into routine production.

The Thermo Scientific TSQ 8000 GC-MS/MS system was built with simplicity as a priority. Whether you are starting with a completely new analysis, transferring a method from a single quadrupole GC-MS to a triple quadrupole GC-MS, or porting a known MRM method from another instrument, the TSQ™ 8000 GC-MS/MS system ensures the fastest route to high performance SRM results.

AutoSRM Studies

AutoSRM is comprised of a three-step process. These steps are called studies:

- Step 1: Precursor Ion Study
- Step 2: Product Ion Study
- Step 3: SRM Optimization Study

Step 1: Precursor Ion Study

The purpose of this first step is to select your precursor ions. To start this study, name your compounds and enter your vial numbers. AutoSRM will signal your TSQ 8000 system to run a full scan analysis on the compounds in your standards.

Along with your chromatographic peak and your product ion spectra, you are presented with a table of the most intense product ion masses to select. If desired, AutoSRM can automatically pick them up for you.

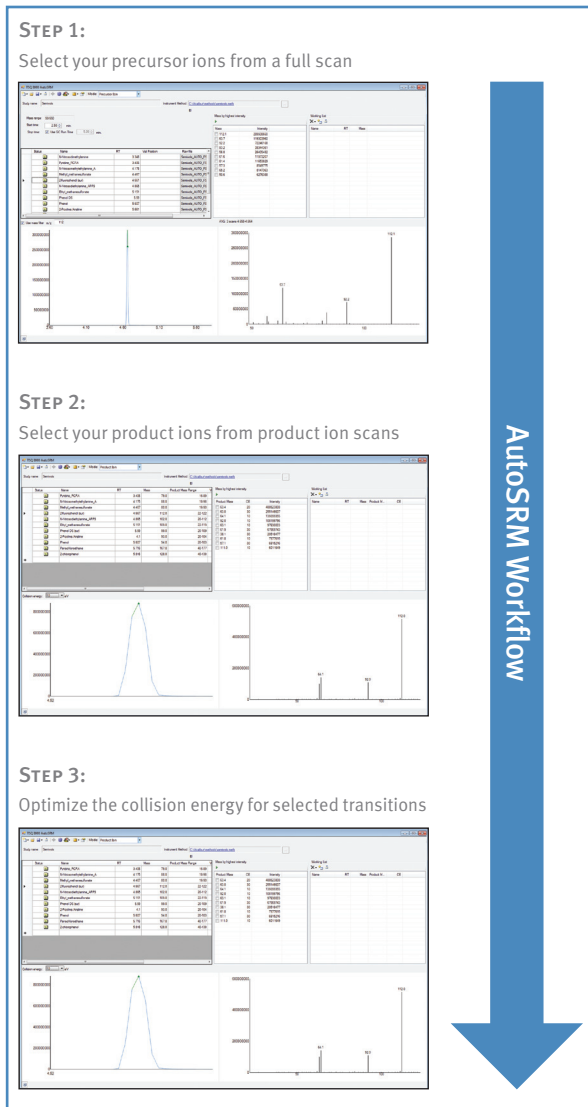

Figure 1. AutoSRM is the first step in creating an SRM method from scratch. AutoSRM can also be used for SIM method development and to optimize an existing SRM method (for instance with a new collision gas).

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Step 2: Product Ion Study

Now that you have selected your precursor masses, it is time to find your product masses. AutoSRM will signal the TSQ 8000 system to acquire product ion scans of your precursor masses at three collision energies. You are not required to set up any methods, sequences or data layouts to accomplish this. AutoSRM automatically takes care of it. Along with your chromatographic peak and your product ion spectra, you are presented with a table of the most intense product ion masses from which to choose. Again, if you choose, you can have AutoSRM pick for you.

From here you can export your transition list to your instrument method, or you can send your selected transitions to an SRM optimization study for further optimization.

Step 3: SRM Optimization Study

The final step in SRM development is the SRM optimization step. Now that you have selected your precursor and product masses, AutoSRM will acquire those transitions at multiple collision energies. Because of the fast scanning capabilities of the TSQ 8000 GC-MS system, three transitions per compound, each at 10 unique collision energies, can be analyzed in a single injection. This will give you a well defined maximum for your collision energy, as shown in Figure 2 below.

Once complete, AutoSRM allows the simple creation of the TSQ 8000 instrument method to be ready for routine analysis.

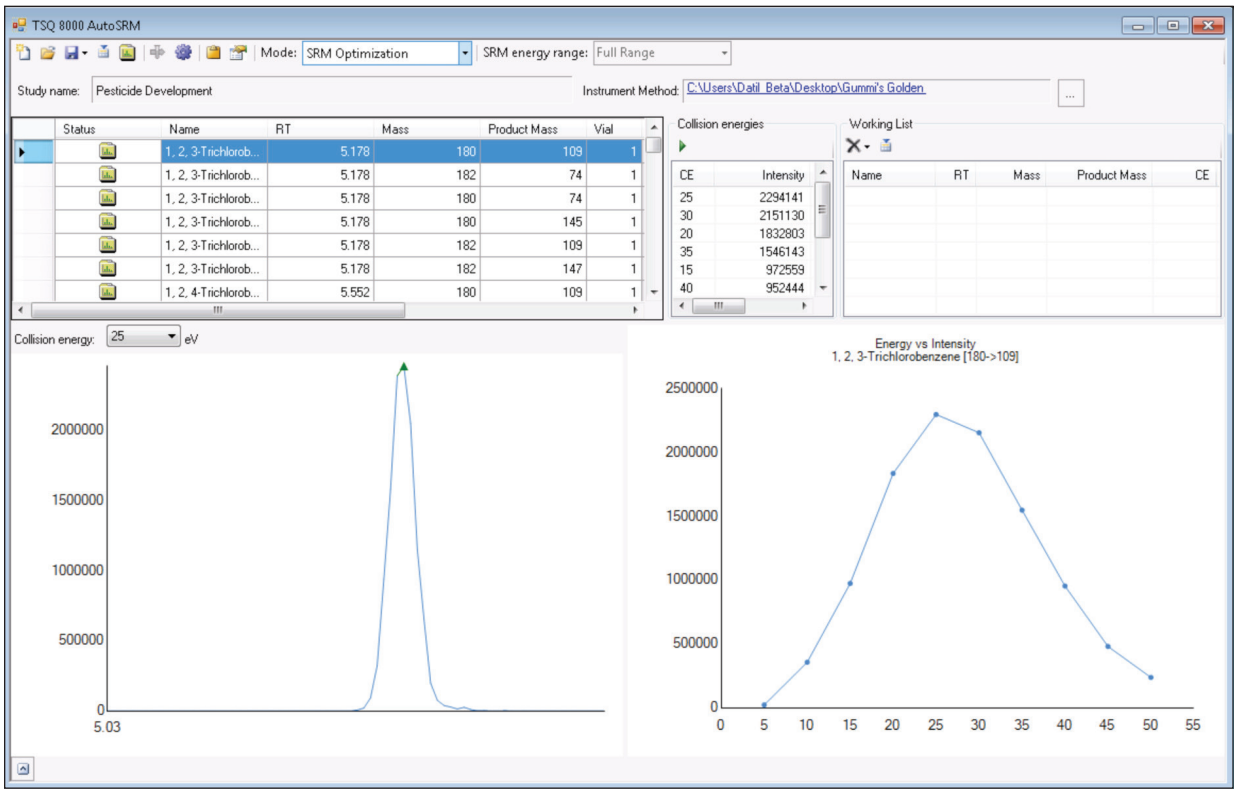

Figure 2. Collision Energy optimization curve for the *m/z* 180 › 109 transition of 1, 2, 3-Tricholobenzene, showing an optimum collision energy of 25 eV. At this point in the process, the optimized SRM transitions can be exported to your instrument method.

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Thermo Scientific TSQ 8000 Triple Quadrupole GC-MS/MS Instrument Method

Introduction

Integration of development, acquisition and processing methods is a key aspect to a truly productive workflow. As shown in the flowchart in Figure 1, the Thermo Scientific TSQ 8000 GC MS/MS instrument method is designed to be fully integrated with two additional primary software applications: AutoSRM and Thermo Scientific TraceFinder software. This enables the convenient migration of compound information from method development to method setup to batch acquisition. The TSQ™ 8000 GC-MS/MS instrument method offers true timed-SRM or timed-SIM acquisition, maximizing instrument sensitivity through instrument efficiency. With additional ease of use and performance features, such as full scan/SRM and scans across the peak checking, performance does not have to be sacrificed for easy operation.

Flexibility and Ease-of-Use

There are many features that make the TSQ 8000 GC-MS/MS instrument method flexible and easy-to-use, such as rich import/export capabilities, true retention time-based acquisition, and scan rate settings that ensure peak sampling rates are sufficient.

Import/Export Functions

The TSQ 8000 GC-MS/MS instrument method allows you to import acquisition lists from common method file types and spreadsheets. Import and export functions are supported with AutoSRM features and TraceFinder™ software. You are not required to enter your acquisition list twice, if you even have to enter it at all, which provides significant time savings.

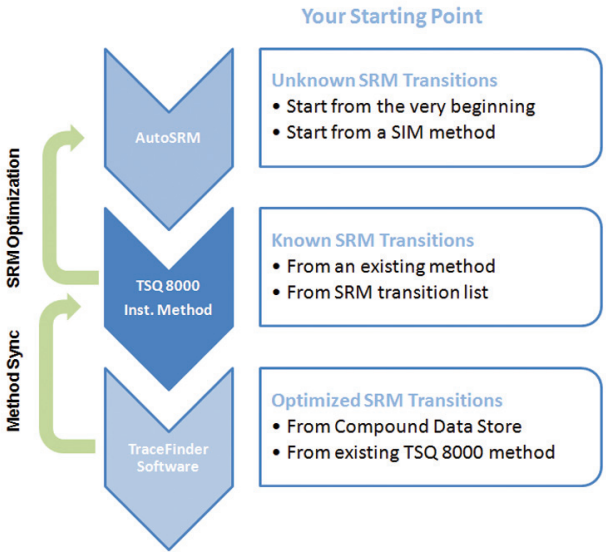

Figure 1. The TSQ 8000 instrument method is fully integrated with AutoSRM and TraceFinder software as part of a complete SRM development and method management workflow.

Retention Time-Based Acquisition

For SIM, SRM, full scan/SIM, or full scan/SRM, the TSQ 8000 GC-MS/MS instrument method allows acquisition both in timed acquisition mode or general acquisition mode. For complex SIM or SRM methods, timed acquisition offers several advantages. One of these advantages is the simplification of method management. For targeted SRM screening applications, it is not uncommon to have 200 or more transitions in a single method. Trying to place such a large number of transitions into discrete segments invariably leads to a handful of transitions that will be very close to a segment end.

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Employing retention time-based acquisition, SIM and SRM windows are set around the retention time of each compound and, therefore, chromatographic peaks are never on the edge of windows (Figure 2), eliminating the risk of missing or only partially acquiring a compound peak due to a small retention time shift. If retention times do shift due to the clipping of the column, for instance, simply update the compound retention time to update acquisition windows.

Scan Rate Settings

To set the acquisition rate of the TSQ 8000 GC MS/MS in timed acquisition mode, simply enter the minimum chromatographic peak width you expect in your method, along with your desired number of data points across the peak, as shown in Figure 3. The instrument method will automatically set the scan rate necessary to achieve this number.

For very complex methods, you might approach the minimum dwell time during the busiest part of the method. If this is the case, the scan event will be flagged. Simply narrow your acquisition windows, or lower your minimum dwell time, to insure that your points across the peak requirement are met.

Maximize Method Performance

To ensure that your methodology is maximizing its performance, the TSQ 8000 GC-MS/MS instrument method allows direct connectivity with AutoSRM and true timed acquisition.

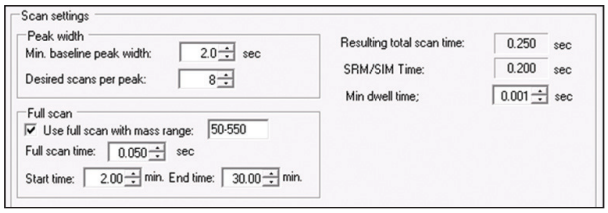

Figure 3. SRM, SIM and full scan rates based on peak width and scans across the peak

Export to AutoSRM

As with the connectivity between the instrument method and TraceFinder software, it is simple to export a transition list from the instrument method to AutoSRM and back again. This allows you to optimize existing transition lists that were not fully optimized before, or were optimized on a different model instrument or with a different collision gas.

Sensitivity and Timed Acquisition

In addition to the ease of use, another advantage of timed-acquisition is that it increases the overall sensitivity for medium to high complexity SRM or SIM methods. With timed acquisition, SRM windows are allowed to overlap (Figure 2), reducing the number of SRM or SIM events taking place at once. This increases average dwell time, which, in turn, increases method sensitivity and precision, allowing for high performance analysis with even the most complex analyte lists.

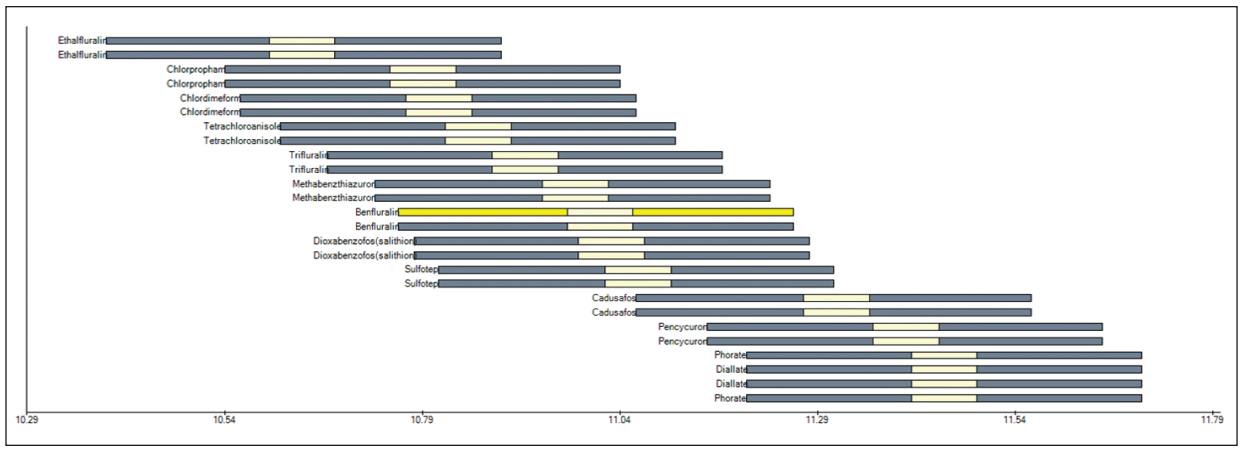

Figure 2. Timed-SRM acquisition list as seen in the TSQ 8000 GC-MS/MS instrument method. Gray bars represent acquisition time for the transitions and yellow bars represent expected compound elution time.

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Thermo Scientific TSQ 8000 GC-MS/MS Method Sync

Introduction

Synchronization of information between various components in the analytical workflow avoids duplication of effort, reduces manual errors, and saves precious time. As shown in the flowchart in Figure 1, the Thermo Scientific TSQ 8000 GC-MS/MS instrument method is designed to be fully integrated with Thermo Scientific TraceFinder software, making it easy to move compound information from method development, to method setup, to batch acquisition. Method Sync enables the user to easily manage complex timed-SRM methods, allowing for a greater focus on acquiring, reviewing, and reporting samples with a smaller focus on maintaining methods.

From Instrument Method to Processing Method

With the TSQ™ 8000 GC-MS/MS system, getting from an instrument method to a full processing method is easy. Within the instrument method, simply export your transition list to a TraceFinder™ Compound Data Store (CDS) file (Figure 2). During this export, you can select which transitions you want to be quantitative and which ones you want to be confirming transitions. After this selection, you can go into the CDS Manager in TraceFinder software, and import your transition list. You can now create your TraceFinder software method, and you can add compounds from the CDS interface to the processing method (Figure 3). The remaining information, such as ion ratios, is updated by associating a data file acquired with the instrument method. All of your target compounds and transitions are now placed within your processing method.

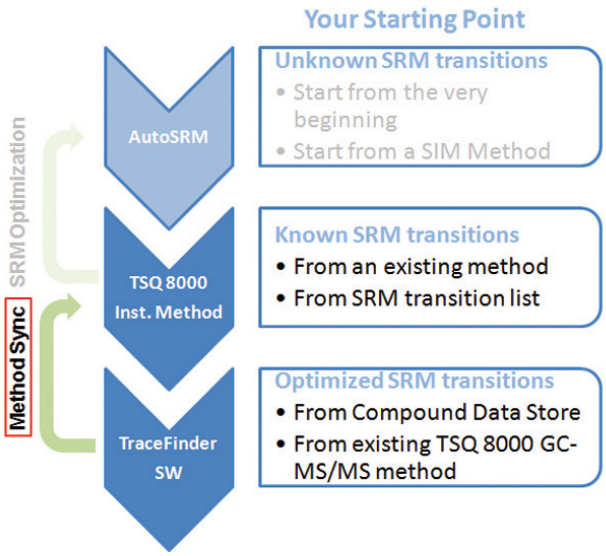

Figure 1. The TSQ 8000 GC-MS/MS instrument method is fully integrated with AutoSRM and TraceFinder software. Method Sync enables TraceFinder software processing method updates to be automatically propagated to the instrument method.

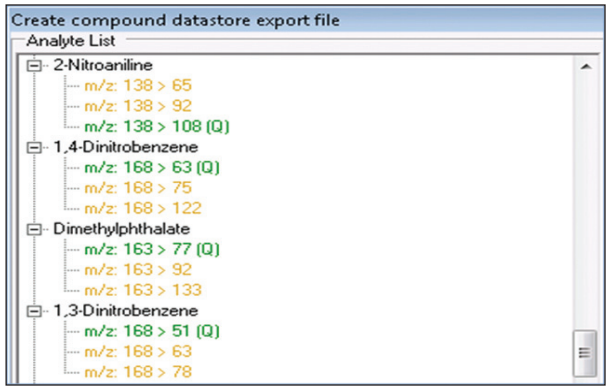

Figure 2. When creating an export file for the TraceFinder Compound Data Store, you are able to select which SRM transitions are for quantitation (shown in green) and which are for confirmation (shown in yellow.)

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Automatic Retention Time Update

Since you now have a retention time-based instrument method to acquire your transitions, and a TraceFinder software method to process the data, how do you accommodate the clipping of your column and all your retention times change? If you sync the TraceFinder method with the acquisition method, all you need to do is run a standard and update your retention times in Data Review (Figure 4). Most of these updated retention times will be determined automatically through automatic peak detection. The next time you run the method, the acquisition list will be updated with the new retention times, eliminating most of the manual work previously needed to maintain a complex SRM instrument method. For these complex methods, this can lead to a major time savings in your daily work.

Compound-Based Scanning

Having the ability to sync the instrument method and the processing method also allows for easy creation of subsets of acquisition lists from the CDS. For instance, if you have a large multi-residue method available, but you are only interested in the organo-chlorine pesticides instead of the full set for a particular analysis, simply select the category for organo-chlorines when creating a processing method from the CDS. This will not only create a processing method with your selected compounds, but it will also create the corresponding acquisition list, limiting the list to just those compounds. This limited transition list will increase the dwell time of the selected transitions, and thus further increase the sensitivity of the TSQ 8000 GC-MS/MS system.

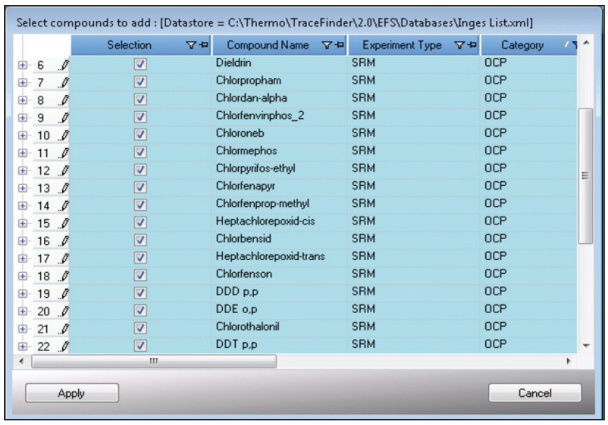

Figure 3. SRM, SIM and full scan rates based on peak width and scans across the peak

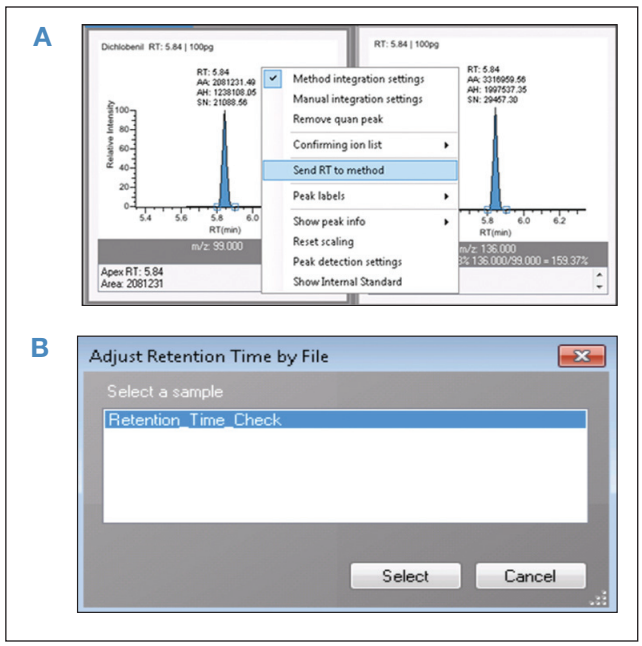

Figure 4. With Method Sync, when you adjust retention times through automatic processing in Data Review, retention times will also be updated in your timed acquisition method. You can update the retention times one at a time while you QC your data in Data Review (a), or adjust retention times en masse from an acquired sample (b).

Chemical Ionization Meets MS/MS Simplicity

The Thermo Scientific TSQ 8000 triple quadrupole GC-MS/MS is built with simplicity as a top priority. Whether you are managing retention times, starting from the beginning with a completely new analysis, transferring a method from a single quadrupole GC-MS, or porting a known MRM method from another instrument, the TSQ™ 8000 GC-MS/MS, through its integrated software tools, ensures the fastest route to high performance results, routinely.

This simplicity allows you to take advantage of the benefits of MS/MS especially when you require more flexibility from your system. Chemical ionization (CI) is an example; sometimes you need a different way of addressing the unique chemistries facing you in a particular application. This less frequently used technique can lead users to feel a little uncertainty in set-up and method development of CI methods. With the TSQ 8000 GC-MS, the uncertainty can be removed; as the system takes care of the critical aspect of method development and set-up, allowing you to proceed with sample analysis.

Where Can CI be Applied in GC-MS/MS?

Generally, the best chance of high sensitivity in MS/MS occurs when the precursor ion can be selected from a single high mass ion which carries a significant amount of the ion current. Electron impact Ionization (EI), although universal, is a high energy process that, in a lot of cases, leads to extensive source fragmentation in less stable compounds. This moves us away for the ideal situation for MS/MS. Since chemical ionization is a softer form of ionization, it offers an opportunity to generate more abundant high mass ions.

CI can also offer a higher degree of selectivity or sensitivity in the source ionization process too, especially with negative chemical ionization (NCI), favoring electronegative compounds such as halogenated species.

Examples for routine analysis using positive chemical ionization (PCI) include applications with target compounds such as phthalates and nitrosamines (US EPA Method 521). PCI is also applied to compound elucidation taking advantage of pseudo molecular ion formation and subsequent MS/MS structural characterization. The obtained data can help screen and confirm any compound candidates obtained through EI spectra and library searching. This is especially useful in conjunction with a direct sample probe, which enables the user to place a sample directly into the source for immediate identification of chemical reaction products.

Applications reported using NCI sometimes include organochloro pesticides (especially pyrethroids), some persistent organic pollutant (POPs) applications like short chain chloropariffins, toxaphenes, and brominated compounds such as polybrominated diphenyl ethers (PBDEs).

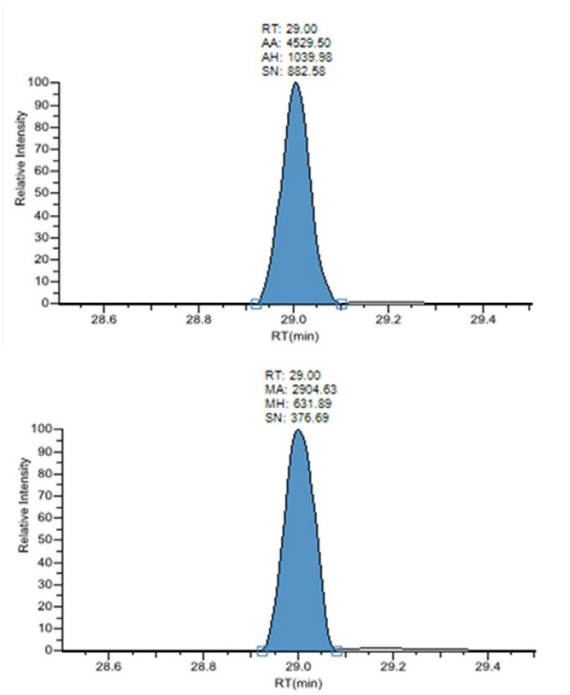

Figure 1. PBDE 209 at 2.5 ppb using NCI on the TSQ 8000 GC-MS System

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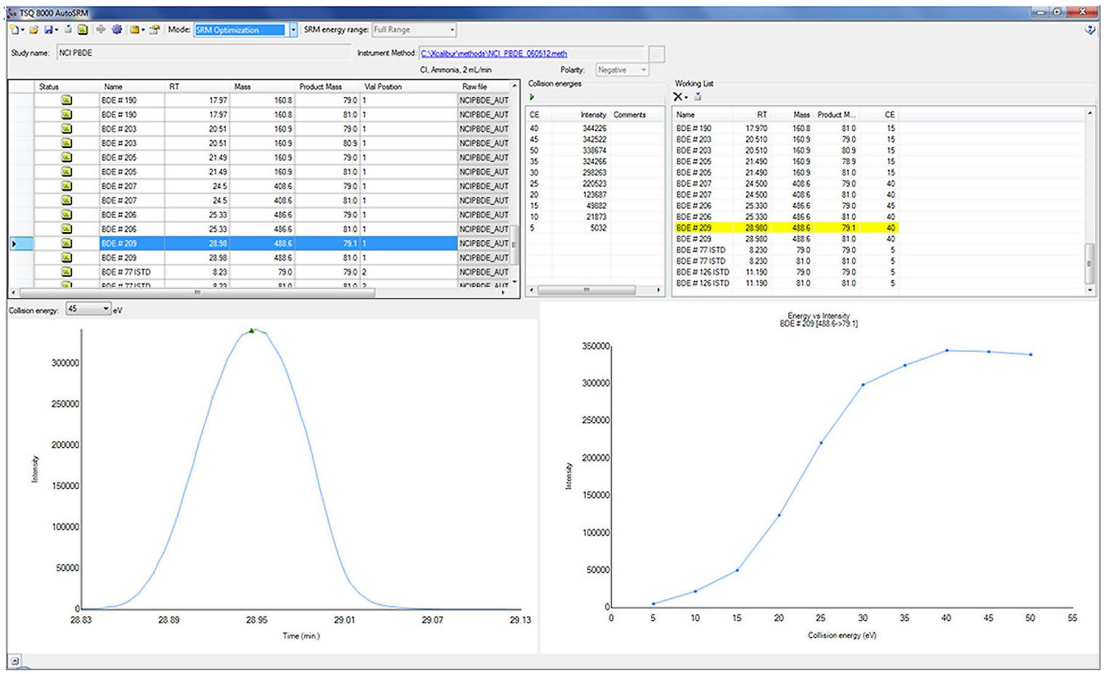

Figure 2. AutoSRM precursor ion selection PBDE

Examples of PBDE data acquired on the TSQ 8000 GC-MS are shown in Table 1 and Figure 1. Also of interest are applications that utilize halogenated derivatized reagents, such as the analysis of estrogenic compounds or tetrahydrocannabinol (THC) in hair.

Table 1. Quantitative performance for PBDEs using NCI SRM on the TSQ 8000 GC-MS System.

MDLs @ 99% Confidence Level n = 8				
PBDE #	Concentration Range Measured (pg/µL)	RSD	R^2 Value	MDL (pg/µL)
47	0.5–250	7%	0.9999	0.106
99	0.5–250	5%	0.9997	0.081
100	0.5–250	8%	0.9995	0.113
153	1-500	5%	0.9996	0.147
154	1-500	9%	0.9997	0.276

CI Method Development

Compared to EI, CI is not as widely used in GC-MS/MS. Consequently, there is not a significant amount of reference information available to help with the setting up of new methods. This technique has the potential to be an unfamiliar and laborious method development process for laboratories. It's especially complex for applications like this, which offers a compelling reason to use the TSQ 8000 GC-MS system.

How the TSQ 8000 GC-MS Can Assist CI Method Development

1. The stages of instrument set-up and method development are all manageable through smart software tools integrated into the workflows needed for both positive and negative mode CI.
2. The TSQ 8000 GC-MS automatically handles CI source tuning and optimization, and offers automated switching between two CI gases.
3. AutoSRM software walks you through the CI method development process to obtained fully optimized SRM transitions (see Figure 2).
4. The TSQ 8000 GC-MS method, linked with AutoSRM, can automatically import developed CI SRMs and optimize MS/MS acquisition for maximum sensitivity through timed-SRM.
5. Thermo Scientific TraceFinder Software methods can also import compound information, control sequences, and quantify target compounds
6. Sample probes, which enable sample placement directly into the source, allow for fast and easy compound characterization of solids or liquids in CI, further facilitated by MS/MS which is available on the TSQ 8000 GC-MS system.

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GC Analysis of Acylated Sugars

Anila I Khan, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words
Derivatization reagent, acylation, n-methyl-bis(trifluoroacetamine) (MBTFA) glucose, fructose, sugars, TR-1701

Abstract
Sugars must be derivatized to a volatile form so as to be analyzed by GC. A commonly used derivatization reagent, N-Methyl-bis (trifluoroacetamide) (MBTFA) was used for converting sugars to their volatile forms. In order to achieve separation of TFA sugar derivatives such as fructose and glucose anomers, a mid-polar 14% cyanopropylphenyl polysiloxane phase GC column was used.

Introduction
Sugars such as glucose and fructose are very difficult to analyse by GC, as they decompose in the injector port and "crash" out on the column. The highly polar and involatile nature of the sugars reduces the efficiency of the detection of these molecules. To overcome these problems the sugars can be derivatized to remove the active hydrogens such as -OH, therefore increasing volatility and improving detectability. The most commonly used derivatization method for the analysis of sugars is an acylation reaction. The Thermo Scientific acylation reagent MBTFA is used for derivatizing sugars and it is manufactured to meet the exacting need of sensitive derivatization reactions. This involves converting the active hydrogen into trifluroesters via a carboxylic derivative. The ester in the derivatized sugar improves the volatility, which makes it easier for analysis by GC/FID MBTFA, like the majority of derivatization reagents, produces a by-product. In this case the formation of the byproduct N-methyltrifluoroacetamide does not interfere with the analysis as it elutes earlier in the chromatogram.

In order to achieve separation of fructose and glucose anomers which arise upon derivatization, a mid-polarity 14% cyanophenyl polysiloxane Thermo Scientific TRACE TR-1701 column was used.

Experimental Details

Sample Preparation
5 mg each of glucose and fructose were weighed into a Thermo Scientific Reacti-Vial containing a Reacti-Vial magnetic stirrer. To the Reacti-Vial, 0.5 mL of MBTFA was added followed by 0.5 mL of Thermo Scientific silylation grade solvent pyridine. The Reacti-Vials were then capped and placed in the Thermo Scientific Reacti-Therm Sample Incubation System and stirred for 1 hour at 65 °C. Once dissolved the reaction was complete. The final sample was then transferred to a 2 mL autosampler vial and 1 µL was injected into the GC/FID.

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Reagents	Part Number
Thermo Scientific MBTFA 10 x 1 mL ampules	TS-49700
Thermo Scientific pyridine silylation grade solvent	TS-27530

Sample Handling Equipment	Part Number
Thermo Scientific Reacti-Therm III Heating/Stirring Module	TS-18823
Thermo Scientific Reacti-Vap III Evaporator	TS-18826
Thermo Scientific Reacti-Block Q-1 (Holds 8 x 10 mL Reacti-Vials)	TS-18814
Thermo Scientific Reacti-Vial clear glass reaction vials 10 mL	TS-13225
Thermo Scientific 2 mL amber vial and screw tops	60180-565

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific TRACE GC Ultra	
Column:	TRACE™ TR-1701 30 m × 0.25 mm × 0.25 µm	260Q142P
Thermo Scientific BTO 17 mm septa		31303211
5 mm ID focus split liner, 105 mm long		453T1905
Graphite liner seal		29033406
10 µL, 50 mm needle length gauge 25 Syringe		36500525
Graphite ferrules to fit 0.1-0.25 mm ID columns		29053488
Carrier gas:	Helium	
Split flow:	60 mL/min	
Column flow:	1.2 mL/min, Constant flow	
Split ratio:	1:1	
Oven temperature:	40 °C (1 min), 10 °C/min, 260 °C (5 min)	
Injector type:	Split/Splitless	
Injector mode:	Split	
Injector temperature:	200 °C	
Detector type:	FID	
Detector temperature:	250 °C	
Detector air flow:	35 mL/min	
Detector Hydrogen flow:	350 mL/min	
Detector nitrogen flow:	30 mL/min	
Thermo Scientific TriPlus Autosampler		
Injection Volume:	1 µL	

Data Processing	
Software:	Thermo Scientific XCalibur

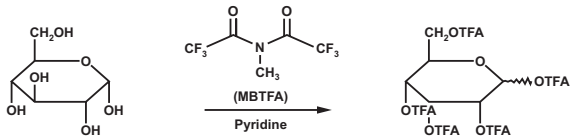

Figure 1: Acylation of glucose with MBTFA and pyridine

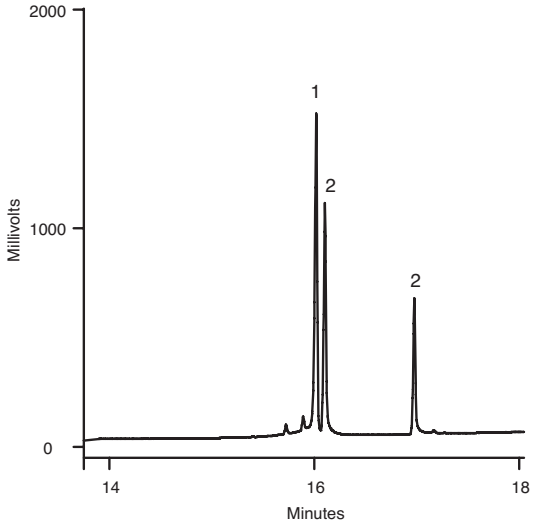

Figure 2: Chromatogram of the separation of derivatized sugars on a TR-1701 column.

Peak Number	Sugar	t_R (min)
1	Fructose	16.0
2	Glucose anomers	16.1 and 17.0

Results

The derivatized glucose produced two peaks, corresponding to the two cyclic forms of glucose existing as anomers (Figure 1) and fructose gave rise to one peak. Good baseline separation between fructose and the two anomers of derivatized glucose was observed (Figure 2) using a 14% cyanophenyl polysiloxane phase column. The stability of the sugars is improved as the acylation reagent protects the unstable groups, aiding separation on the chromatographic column.

Conclusion

MBTFA is an ideal derivatization reagent for increasing the volatility of sugars. This enabled enhanced separation and detection of fructose and glucose anomers using a TRACE TR-1701 GC column.

References

Thermo Scientific reagents, solvents and accessories brochure (Ref: BR20535_E06/12). Available upon request. ACD labs software to draw chemical structures.

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Thermo Scientific TraceGOLD GC Columns offer you a leap forward in column performance, delivering ultra low bleed, superior inertness, and the highest level of reproducibility, guaranteed.

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- Runs the chromatography, processes the data, and produces final results

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Technical Note 52364

Analytical Repeatability, Accuracy, and Robustness of Instant Connect GC Modules

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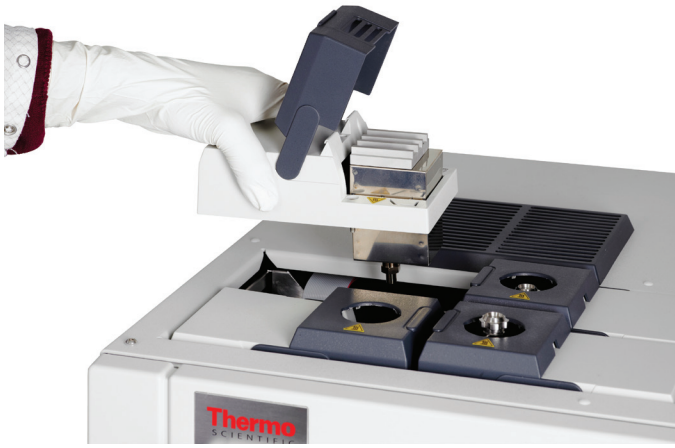
Key Words
TRACE 1300 Series GC, Instant Connect modules, SSL, PTV, FID, ECD, TCD, NPD

Introduction

GC injectors and detectors are considered fundamental components of a gas chromatography system. In modern instrumentation, they consist of the mechanical parts, the inner body with all tubing for gas connections, pneumatics, and electronic controls. Selection of an appropriate injector and detector is based on application requirements. Changing a system configuration to follow a new analytical need or application is a complex operation, requiring specialized service assistance and often resulting in a new system requirement.

The Thermo Scientific™ TRACE™ 1300 Series GC is the first GC instrument on the market that has transcended this design model. Similar to the long-established modularity in HPLC, it makes the fundamental instrument components (the injector and detector) available as independent sub-systems, which are combined to produce the desired analytical layout. The level of modularity of the TRACE 1300 Series GC allows users to rapidly adapt the instrument configuration to new application and/or workload requirements without consulting a service engineer.

This new GC modularity is implemented in the TRACE 1300 Series GC in the form of a full range of injector and detector modules, which are easy and quick to install and swap. These modules, termed *Instant Connect*, incorporate all relevant pneumatic hardware and electronic parts necessary for making the injector or the detector a fully self-sufficient sub-unit of the instrument. All electronic circuits and pneumatic controls are integrated into the injector body or detector cell, and enclosed into a light, 17 cm x 10 cm x 6 cm, easy-to-handle housing. Each module stores all specific electronic and pneumatic calibration information, minimizing module-to-module performance variation. The modules are plugged into the top part of the GC, are automatically configured into the system, and connected to the gas supply lines. Installing a module takes only two minutes: the time needed to fix three retaining screws and slide the new injector or detector module in place.



Laboratories can benefit from the versatility provided by this “Instant Connect” modularity in several ways:

- Expanding instrument capability at any time, by adding a new injector or detector module to run a new method
- Upgrading a GC from single to multiple channels to satisfy rapid incremental business needs and enhance laboratory productivity
- Replacing contaminated injectors or detectors quickly with clean ones and running samples in a few minutes, while conducting full maintenance and cleaning when the laboratory schedule allows
- Sharing injectors and detectors with different TRACE 1300 Series GC units in a lab depending on the application

The list of “Instant Connect” modules includes Split/Splitless (SSL) and Programmable Temperature Vaporizing (PTV) injectors both in the standard and backflush configuration and all standard GC detectors: Flame Ionization Detector (FID), Electron Capture Detector (ECD), Thermal Conductivity Detector (TCD), and Nitrogen Phosphorous Detector (NPD).

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This technical note describes results obtained from an endurance mechanical test performed on "Instant Connect" modules and typical analytical reproducibility and accuracy provided by the TRACE 1300 Series GC. Analytical reproducibility was also measured after switching modules of the same type in the same application, as might happen when an injector module is replaced with a new one in a routine laboratory to keep a contaminated instrument up and running.

Experimental

A TRACE 1310 GC instrument equipped with various SSL and FID modules was used in all of the experiments, and all "Instant Connect" modules are identified by specific serial numbers for easier tracking in the lab. Four different modules were alternated in these experiments.

The GC was equipped with a Thermo Scientific AS 1310 liquid autosampler. All tests were performed using a synthetic mix of normal alkanes ranging from C_{10} to C_{40} in hexane, at a concentration of approximately 10 ppm (10 ng/µL) and using helium as carrier gas. A 1 µL aliquot of sample was injected in splitless mode into a standard glass-wool packed tapered liner, while the injector temperature was maintained at 300 °C. Splitless time was 0.8 minutes. The FID detector temperature was set to 350 °C.

A Thermo Scientific TraceGOLD™ column TR-5, 15 m x 0.25 mm id x 0.25 µm, was used in all experiments. Oven temperature was set at 50 °C for 0.5 min and then ramped up to 340 °C at 20 °C/min, with two minutes of isothermal time at the final temperature. A Thermo Scientific Dionex™ Chromeleon™ Chromatography Data System was used for setting all method parameters, data acquisition, and data processing.

Results and Discussion

System analytical reproducibility and accuracy

The analytical reproducibility was evaluated using two new "Instant Connect" modules, a SSL injector (module serial number S/N: 712100036) and a FID detector (S/N: 712300088), by injecting the synthetic hydrocarbon mix automatically (ten repetitions). Results in terms of peak area and retention time repeatability are summarized in Tables 1 and 2. No discrimination for both volatile and high boiling compounds was seen. As shown in Table 1 the recovery, calculated using C_{20} peak area as reference, is close to 100% along the full range of volatility. Absolute peak area relative standard deviation is far below 1% for all hydrocarbons. All injector and detector modules incorporate a new generation of miniaturized gas controls. These integrated electronic devices ensure precise control of the inlet pressure and the flow throughout the column, further contributing to the excellent reproducibility of retention times. As indicated in Table 2, the standard deviation is below a thousandth of a minute. This level of reproducibility is a clear indication of the accurate temperature profile and column flow maintained during the ramp and the precise thermo-regulation of the GC oven. Overall results show full recovery of hydrocarbons and excellent data precision.

Table 1. Instrument configuration SSL S/N 712100036 and FID S/N 712300088. Absolute peak area RSD% far lower than 1%. Recovery, measured as ratio vs C_{20} average area, at 100% for the whole range of hydrocarbons

SSL 712100036 / FID 712300088	nC10	nC12	nC14	nC16	nC18	nC20	nC22	nC24	nC26	nC28	nC30	nC32	nC34	nC36	nC38	nC40
Injection 1	2600304	2647767	2600941	2619188	2552750	2565809	2543886	2535687	2512699	2527008	2602759	2597807	2534441	2564855	2470270	2525384
Injection 2	2610605	2657856	2601653	2623404	2568557	2579380	2565938	2565679	2545232	2560614	2636265	2629734	2557462	2596729	2495209	2563483
Injection 3	2602666	2653832	2599714	2626029	2553641	2577265	2561577	2558672	2542703	2555406	2632496	2630095	2555743	2580475	2480864	2538819
Injection 4	2636572	2683702	2632897	2656448	2593709	2602685	2584957	2575384	2558834	2574920	2649582	2640082	2566623	2593858	2490053	2549873
Injection 5	2623737	2668679	2617130	2639475	2575209	2588255	2568857	2566489	2551218	2570336	2641759	2632243	2559472	2591033	2487269	2545848
Injection 6	2628675	2671731	2625320	2647746	2586155	2602674	2584119	2578956	2563433	2577945	2652932	2644762	2572449	2600568	2495549	2560371
Injection 7	2633245	2675436	2621623	2640507	2579749	2601553	2603546	2589030	2566470	2580193	2651340	2644782	2575086	2615870	2515378	2552861
Injection 8	2622426	2667773	2618401	2631007	2571368	2588047	2571982	2568771	2543992	2565899	2635937	2628421	2556233	2599156	2491820	2552234
Injection 9	2627383	2675413	2624978	2646945	2578061	2590137	2578171	2582555	2553973	2565982	2637795	2636002	2561603	2598494	2504800	2575965
Injection 10	2621650	2664829	2611668	2634863	2576839	2592681	2577082	2571091	2552087	2567396	2634338	2631176	2560023	2590260	2497076	2558360
Average (counts)	2620726	2666702	2615432	2636561	2573604	2588848	2574011	2569231	2549064	2564570	2637520	2631510	2559913	2593130	2492829	2552320
SD	12355	10941	11571	11889	12894	12092	15913	14767	15078	15223	14298	13319	11161	13430	12358	13908
RSD %	0.47%	0.41%	0.44%	0.45%	0.50%	0.47%	0.62%	0.57%	0.59%	0.59%	0.54%	0.51%	0.44%	0.52%	0.50%	0.54%
Recovery %	101%	103%	101%	102%	99%	100%	99%	99%	98%	99%	102%	102%	99%	100%	96%	99%

Module-to-module reproducibility

To simulate a situation where a laboratory needs to quickly replace a module, such as to avoid interrupting instrument throughput for maintenance, the "Instant Connect" SSL injector module (S/N: 712100036) was replaced by a new module (S/N: 712300021). This required cooling and powering down the instrument, disconnecting the column from the original SSL injector module, removing the module and plugging in the new one, connecting the column, and powering up the TRACE 1310 GC again. Electronic gas control permits an automated leak check to be performed to guarantee that no artifacts are introduced by this manual operation. The reduced thermal mass GC design allows a quick recovery of injection-ready conditions after instrument power-up. As a result, the GC was ready to resume analytical injections again in only nine minutes after it was originally powered down. A blank GC cycle was programmed before injecting samples again, which is good practice to ensure the entire flow path was not affected by air introduced during module replacement.

An automated sequence of 10 injections was performed immediately after the module replacement along with collecting data. The instrument was then stopped again, and the FID detector module (S/N: 712300088) was replaced by a new one (S/N: 712300126). After a blank run, another sequence of 10 injections completed the experiments. Tables 3 and 4 and Figure 1 summarize the repeatability results for the three different instrument configurations. Variations in peak area measured as a delta of the average counts are in the range of a few percentages when changing either the injector or the FID detector. Such a variation, for many applications, is well below the required limit of a system suitability check, eliminating the need to recalibrate the GC system as a whole. The retention time variations are in the range of a few hundredths of a minute or even less with no impact on component retention time.

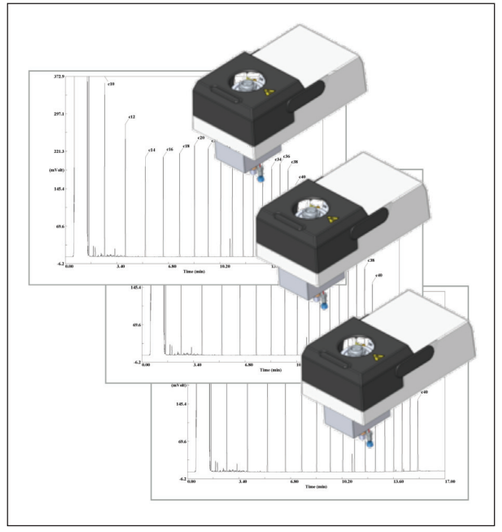

Figure 1. Module-to-module repeatability. Modules store all of their calibration information allowing minimum variation if replaced on a system.

Table 2. Retention time standard deviation in the range of 1/1000 minute

	nC10	nC12	nC14	nC16	nC18	nC20	nC22	nC24	nC26	nC28	nC30	nC32	nC34	nC36	nC38	nC40
Injection 1	2.562	3.935	5.253	6.445	7.525	8.506	9.408	10.237	11.005	11.717	12.385	13.010	13.598	14.153	14.680	15.190
Injection 2	2.562	3.933	5.252	6.445	7.525	8.508	9.408	10.237	11.005	11.718	12.385	13.010	13.598	14.153	14.678	15.188
Injection 3	2.562	3.933	5.252	6.445	7.523	8.505	9.407	10.233	11.003	11.715	12.383	13.007	13.597	14.152	14.680	15.189
Injection 4	2.562	3.935	5.253	6.445	7.525	8.508	9.408	10.237	11.005	11.718	12.385	13.008	13.598	14.152	14.678	15.188
Injection 5	2.562	3.933	5.252	6.445	7.525	8.508	9.408	10.237	11.003	11.717	12.385	13.010	13.600	14.154	14.678	15.191
Injection 6	2.562	3.933	5.252	6.445	7.525	8.508	9.408	10.237	11.005	11.717	12.385	13.010	13.598	14.153	14.680	15.189
Injection 7	2.562	3.933	5.252	6.447	7.525	8.507	9.407	10.237	11.003	11.718	12.385	13.008	13.597	14.153	14.681	15.190
Injection 8	2.562	3.933	5.252	6.445	7.525	8.507	9.408	10.235	11.003	11.717	12.385	13.008	13.598	14.150	14.680	15.190
Injection 9	2.560	3.932	5.250	6.443	7.523	8.506	9.408	10.235	11.002	11.717	12.385	13.008	13.597	14.153	14.682	15.191
Injection 10	2.562	3.933	5.252	6.445	7.525	8.508	9.408	10.237	11.005	11.718	12.385	13.010	13.597	14.152	14.682	15.188
Average (minutes)	2.562	3.934	5.252	6.445	7.525	8.507	9.408	10.236	11.004	11.717	12.385	13.009	13.598	14.153	14.680	15.190
SD	0.0005	0.0009	0.0009	0.0008	0.0007	0.0012	0.0007	0.0012	0.0012	0.0011	0.0005	0.0012	0.0011	0.0012	0.0013	0.0010
RSD %	0.02%	0.02%	0.02%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.00%	0.01%	0.01%	0.01%	0.01%	0.01%

Table 3. Variation in peak area as effect of module swap. All variations are in the range of few % changing either the inlet or the FID detector

Original instrument configuration SSL s/n 712100036 and FID s/n 712300088																
	nC10	nC12	nC14	nC16	nC18	nC20	nC22	nC24	nC26	nC28	nC30	nC32	nC34	nC36	nC38	nC40
Average (counts)	2620726	2666702	2615432	2636561	2573604	2588848	2574011	2569231	2549064	2564570	2637520	2631510	2559913	2593130	2492829	2552320
SD	12355	10941	11571	11889	12894	12092	15913	14767	15078	15223	14298	13319	11161	13430	12358	13908
RSD %	0.47%	0.41%	0.44%	0.45%	0.50%	0.47%	0.62%	0.57%	0.59%	0.59%	0.54%	0.51%	0.44%	0.52%	0.50%	0.54%

Change of SSL module - Instrument configuration SSL 712300021 / FID 712300088																
Average (counts)	2705439	2722254	2654680	2680682	2615418	2647035	2626550	2624551	2604909	2618663	2699958	2707570	2658013	2713142	2598635	2604178
SD	8276	7559	8759	9119	11059	11146	12635	14822	13711	16916	16529	17096	12977	10030	12448	10215
RSD %	0.31%	0.28%	0.33%	0.34%	0.42%	0.42%	0.48%	0.56%	0.53%	0.65%	0.61%	0.63%	0.49%	0.37%	0.48%	0.39%
Variation %	-3.2%	-2.1%	-1.5%	-1.7%	-1.6%	-2.2%	-2.0%	-2.2%	-2.2%	-2.1%	-2.4%	-2.9%	-3.8%	-4.6%	-4.2%	-2.0%

Change of FID module - Instrument configuration SSL 712300021 / FID 712300126																
Average (counts)	2752208	2777431	2705697	2728377	2668020	2699389	2678126	2670723	2649792	2665081	2745907	2757795	2703327	2763143	2653118	2666225
SD	13455	15147	15120	11600	15162	14201	15885	15954	14781	15601	11514	14864	10635	13223	15755	11218
RSD %	0.49%	0.55%	0.56%	0.43%	0.57%	0.53%	0.59%	0.60%	0.56%	0.59%	0.42%	0.54%	0.39%	0.48%	0.59%	0.42%
Variation %	-1.7%	-2.0%	-1.9%	-1.8%	-2.0%	-2.0%	-2.0%	-1.8%	-1.7%	-1.8%	-1.7%	-1.9%	-1.7%	-1.8%	-2.1%	-2.4%

Table 4. Variation in retention time as effect of module swap. All variations are in the range of 1/100 of a minute or less, changing either the inlet or the FID detector

Original instrument configuration SSL s/n 712100036 and FID s/n 712300088																
	nC10	nC12	nC14	nC16	nC18	nC20	nC22	nC24	nC26	nC28	nC30	nC32	nC34	nC36	nC38	nC40
Average (minutes)	2.562	3.934	5.252	6.445	7.525	8.507	9.408	10.236	11.004	11.717	12.385	13.009	13.598	14.153	14.680	15.190
SD	0.0005	0.0009	0.0009	0.0008	0.0007	0.0012	0.0007	0.0012	0.0012	0.0011	0.0005	0.0012	0.0011	0.0012	0.0013	0.0010
RSD %	0.02%	0.02%	0.02%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.00%	0.01%	0.01%	0.01%	0.01%	0.01%

Change of SSL module - Instrument configuration SSL 712300021 / FID 712300088																
Average (minutes)	2.566	3.938	5.255	6.448	7.527	8.509	9.410	10.238	11.005	11.719	12.386	13.011	13.599	14.154	14.679	15.188
SD	0.0006	0.0012	0.0007	0.0004	0.0009	0.0007	0.0007	0.0014	0.0007	0.0015	0.0009	0.0015	0.0009	0.0014	0.0015	0.0014
RSD %	0.02%	0.03%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
Variation %	-0.2%	-0.1%	-0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Change of FID module - Instrument configuration SSL 712300021 / FID 712300126																
Average (minutes)	2.563	3.935	5.254	6.446	7.525	8.508	9.408	10.237	11.004	11.718	12.384	13.011	13.598	14.154	14.679	15.186
SD	0.0007	0.0007	0.0010	0.0007	0.0014	0.0008	0.0009	0.0014	0.0010	0.0009	0.0009	0.0010	0.0018	0.0007	0.0014	0.0019
RSD %	0.03%	0.02%	0.02%	0.01%	0.02%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
Variation %	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Injector module endurance test

Module robustness was tested mechanically by having ten different operators repeatedly insert and remove the module. The operators had widely varied skills and knowledge, with some subjects having no prior GC experience. Two operators were from the shipping department, and two worked in order processing. Two had limited GC knowledge and were the Quality Manager and Product Manager of a different product line. Finally, two engineers and two GC scientists that also presided over all of the tests participated. The module subjected to the test was the "Instant Connect" SSL injector (S/N: 712300021), and the sequence applied by each operator included powering off the GC, removing the module, inserting the module, and powering up the GC until it reached stand-by condition. The total average test sequence time was six minutes. Each operator repeated this cycle ten times.

After each operator finished his or her cycle, the column was connected again to the SSL injector and FID detector, followed by a double blank run. Ten automated injections of the hydrocarbon mix completed the test. Tables 5 and 6 include the results of the last two runs performed before starting the ruggedness test and the two initial runs of the new sequence. It is useful to note that the new sequence was started the day after the last sequence of injections was recorded. The variations of both absolute peak areas and retention times indicate the module performed perfectly without requiring any maintenance.

Table 5. Variation in peak area before and after 100 times module replacement cycle

	nC10	nC12	nC14	nC16	nC18	nC20	nC22	nC24	nC26	nC28	nC30	nC32	nC34	nC36	nC38	nC40
Injection 9 before IC swap	2754987	2779540	2709468	2720590	2662466	2694642	2671418	2666034	2640542	2660383	2748956	2756412	2705301	2768808	2658421	2670870
Injection 10 before IC swap	2751265	2775027	2708032	2732281	2677453	2705799	2688053	2684329	2667261	2684684	2755387	2771243	2709754	2772642	2651536	2665536
Injection 1 after IC swap	2767372	2791927	2719553	2738439	2664499	2693367	2672357	2657758	2643338	2655810	2738028	2745997	2704789	2768416	2664390	2670998
Injection 2 after IC swap	2756768	2787601	2711585	2738364	2687682	2720242	2699762	2690563	2663741	2677520	2756966	2774421	2711745	2765971	2664631	2676359
Variation	-0.59%	-0.61%	-0.43%	-0.23%	0.48%	0.46%	0.58%	0.99%	0.90%	1.08%	0.63%	0.91%	0.18%	0.15%	-0.48%	-0.20%

Table 6. Variation in retention time before and after 100 times module replacement cycle

	nC10	nC12	nC14	nC16	nC18	nC20	nC22	nC24	nC26	nC28	nC30	nC32	nC34	nC36	nC38	nC40
Injection 9 before IC swap	2.562	3.935	5.253	6.447	7.525	8.507	9.408	10.235	11.003	11.717	12.383	13.010	13.598	14.153	14.678	15.185
Injection 10 before IC swap	2.563	3.933	5.255	6.445	7.523	8.508	9.407	10.237	11.005	11.717	12.383	13.012	13.595	14.154	14.677	15.185
Injection 1 after IC swap	2.563	3.935	5.253	6.447	7.525	8.507	9.408	10.238	11.003	11.718	12.385	13.010	13.598	14.153	14.678	15.183
Injection 2 after IC swap	2.563	3.935	5.253	6.447	7.523	8.508	9.407	10.237	11.003	11.718	12.385	13.012	13.600	14.155	14.680	15.187
Variation	-0.03%	-0.04%	0.04%	-0.03%	-0.02%	0.01%	-0.02%	-0.02%	0.02%	-0.01%	-0.01%	0.02%	-0.02%	0.00%	-0.01%	0.01%

Conclusion

The “Instant Connect” modules on the TRACE 1300 Series GC offer important advantages over conventional GC instrumentation, such as maintaining instrument uptime and continuing to run even when an injector or detector must be replaced for maintenance purposes. Additional advantages include the ability to upgrade from a single-channel GC to a double-channel GC to increase instrument productivity and the option to add a new detector module to respond to new application requirements.

The design of “Instant Connect” modules as self-independent components of the GC, which incorporate all mechanical and electronic components with calibration information, permits the user to rapidly remove and install new modules without any service assistance. The modularity of the design provides configuration flexibility never before available in a GC and also maintains the highest reproducibility and ruggedness standards. The test results show that module internal calibration allows module-to-module reproducibility to be within 5% of the variances in absolute peak area and retention times. The compact size and robustness of the module design enable the modules to be repeatedly replaced without impacting instrument performance.

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U-SRM – Ultra Selective Detection of Analytes in Complex Matrix Samples on the TSQ Quantum XLS Ultra GC-MS/MS

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Key Words
GC-MS/MS, U-SRM, Mass Resolution, Matrix, Selectivity, TSQ Quantum XLS Ultra

Introduction

The Thermo Scientific TSQ Quantum XLS Ultra triple quadrupole mass analyzer breaks the barrier of low level matrix interferences for a reliable compound quantitation. Where single quadrupole analyzers are limited to isobaric interferences on the same selected ion monitoring mass (SIM), a triple quadrupole mass spectrometer offers increased matrix selectivity. With this, triple quadrupole detection has provided a large step forward in our ability to detect at very low concentrations in complex matrix. But, the selectivity of this nominal mass analyzer can be challenged when some matrix/target combinations are considered. When analyzing at extremely low concentrations, the overwhelming intensity of matrix compounds can still provide interferences in some cases, as chemical noise, on the product ion mass traces. It is not surprising in this context that different matrices interfere with different impact. So, what is the technical solution the TSQ Quantum XLS Ultra™ triple quadrupole GC-MS/MS system is using to break these barriers and overcome these typical matrix limitations?

Delivering High Performance Triple Quadrupole Experiments

The TSQ Quantum XLS Ultra, as shown in Figure 1, provides enhanced mass resolution capabilities that are unique to GC-triple quadrupole systems. The specially designed high precision hyperbolic quadrupoles (Thermo Scientific HyperQuad quadrupoles), as pictured in Figure 2, provide enhanced mass resolution and ion transmission. This increased analyzer performances allows an extremely low background signal which simplifies quantification of trace compounds even when shadowed by hugely intense matrix components, of which a significant portion exhibit isobaric ions interfering with the selected reaction monitoring (SRM) process. For trace analysis in the ppt range, the increased radius of the HyperQuad™ quadrupole assembly used a 50% larger diameter between the rods for increased ion transmission and hence sensitivity.

Of course, high performance quadrupoles are a pre-requisite for a good GC triple quad system as the mass analyzers lie at the heart of the performance delivery. In a triple quadrupole analyzer, the first quadrupole (Q1) that sits directly behind the ion source and pre-filter, has a large influence on the target analyte selectivity of the mass spectrometer. The desired precursor ions, after being generated in the ion source, are selected by Q1 and are transmitted to the collision cell (Q2) for fragmentation. The third quadrupole (Q3) is set to transmit to the detector only preselected products ions generated in Q2 as a result of collision induced fragmentation (CID).

Without high-performing quadrupoles, mass selection capabilities become compromised and tend towards a lower resolution to transmit the same number of ions. Systems that do not employ HyperQuad technology have to open the mass transmission window (above unit mass resolution) to transmit the same number of ions as a HyperQuad analyzer in unit mass mode. This decreased selectivity for target ions can give rise to chemical

Figure 1. TSQ Quantum XLS Ultra enhanced mass resolution GC-MS/MS system

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interferences in the analysis. The same effect can be observed when mass resolution settings of 1.2 Da FWHM or higher are used during single quadrupole analysis. This can be seemingly effective when simple sample matrices are encountered. However, when facing a more complex sample matrix this strategy can cause problems in the analysis.

What are Isobaric Interferences?

The term "isobaric interferences" describes ions of the same nominal mass but of different chemical composition and structure. The "nominal mass" is typically the mass used in quadrupole instruments for programming SIM or SRM acquisition. This reflects the typical unit mass resolution capabilities of standard quadrupole instruments. The nominal mass, in this context, can be described as a 1 Da (1 *m/z*) resolution capability between mass peaks. The term "isobaric interference" means in practice that ion signals from other compounds than the target analyte appear at the same nominal mass in the scan spectrum, the SIM trace or, in some cases, the SRM trace.

Figure 2. HyperQuad quadrupole rods used in the TSQ Quantum XLS Ultra system

For triple quadrupole instruments operated in SRM mode, the selectivity is generally high. This is due to the MS/MS process. There are cases, however, where despite MS/MS being applied, selectivity is challenged. These cases appear more frequently when the matrix load of samples is very high. This is not unusual for a triple quadrupole, as often the most complex quantitative determinations are directed to this type of technology. The likelihood of encountering a full SRM interference increases as a function of the matrix complexity. The observed effects of isobaric interferences are also more apparent when targeting compounds in low or sub-ppb concentration ranges. This is because the target compound mass is more likely to be "shadowed" by interfering matrix ions (especially in the first stage of MS in Q1) that are typically orders of magnitude higher in concentration. The drive towards shorter clean-up procedures also pushes additional matrix to the detection system, adding to the problem.

When these intense interfering matrix ions successfully transmit through Q1 into the collision cell, there is a higher statistical probability that interfering product ion masses are formed. This gives rise to a higher occurrence of full SRM interferences and visibly reduced analyte selectivity. This often manifests as an increased chemical noise background and hence, low signal-to-noise detection. This is observed most frequently in matrix samples and is often unnoticed or absent in solvent only standards (see Figure 3). Because solvent standards are relatively clean, it is also possible (and sometimes practiced) to open Q1 above unit mass resolution (so called "wide" or "open" settings.) This creates the possibility to gain sensitivity, but does not help much when considering real backgrounds in complex samples. Sensitivity (and selectivity) achieved at wider Q1 resolution values can vary considerably between clean and dirty samples. With that in mind, it is sensible that any comparisons between instruments, especially those that are to face dirtier samples, are performed in matrix samples. If solvent standards are to be used, then the true instrument sensitivity should be compared using equivalent Q1 and Q3 resolution values.

The observed effects of isobaric interferences are also more apparent when targeting compounds in low or sub-ppb concentration ranges.

How Triple Quadrupole Analyzers Work

The idea of using three quadrupoles arranged in series in a triple quadrupole analyzer for structure elucidation ("an added dimension of mass spectral information") follows an idea first reported by Richard Yost and Chris Enke of Florida University in 1970. The analyzer should allow the detection of structure-related information and overcome the single quadrupole limitation of measuring a mass

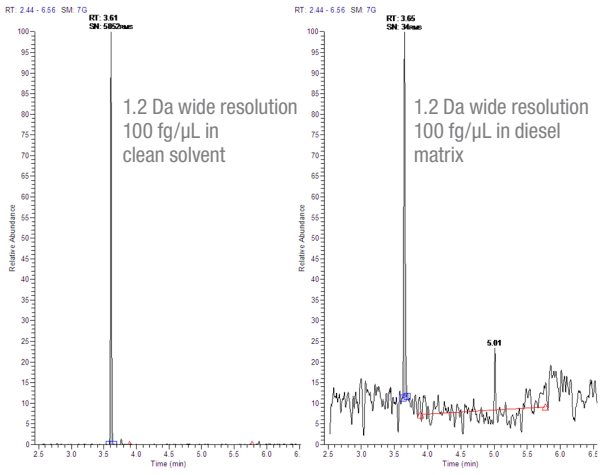

Figure 3. High occurrence of matrix interference on standard specification compound (octafluoronapthalene) with "Wide/Open" Q1 settings (1.2 Da FWHM) absent in solvent standard (left) but significant when in presence of complex matrix (right).

"only," working as a mass selective detector. The new capabilities have revolutionized all structure elucidation work at a time before quantitative triple quadrupole applications appeared.

Today, standard triple quadrupole analyzers still deliver selectivity through the same structure related mechanism. Advanced systems, such as the TSQ Quantum XLS Ultra, deliver selectivity via two mechanisms: structure-selective detection and enhanced mass resolution capabilities.

Structure-Selective Detection

The chemical composition and structure of a molecule determines the specific pathways of ion source fragmentation during ionization. During this process, energy is transferred to a compound, and it is distributed throughout the molecule and eventually breaks chemical bonds. This results in a spectrum of different mass fragments and relative intensities. A similar mechanism applies to collision induced dissociation (CID) in the collision cell (Q2) of a triple quadrupole analyzer.

This can be best illustrated having a closer look at a regular EI spectrum, for instance from the well known pesticide parathion. The parathion EI spectrum in Figure 4 shows the intense molecular ion as the base peak (nominal mass 291 *m/z* and accurate mass 291.03 *m/z*) and the dissociation of the molecular ion structure into a number of lower mass fragments, all of them contributing to a fingerprint of the given structure. For a low level SIM detection, the intense molecular ion 291 *m/z* is the signal of choice. When performing SRM on a triple quadrupole instrument the same 291 *m/z* is targeted as the precursor ion. After CID fragmentation in the collision cell, structurally selective product ions are formed and monitored using Q3. In the case of parathion, the product ions 109 and 97 *m/z* (which also occur during EI source ionization – Figure 4) are monitored. These product ions are formed in consistent ratios to each other.

Only structures eluting at the retention time of parathion with a parent ion of 291 *m/z* are expected to give a signal at the product ions 109 and 97 *m/z*. This filters out most of the unspecific background interference at 291 *m/z*, which limits the SIM detection in a single quadrupole instrument.

Adding Mass Selectivity with Enhanced Mass Resolution

In some matrices, the high structural selectivity of a triple quad analyzer can be impaired by a compromised selection of the precursor ion at Q1. The unique capability of the TSQ Quantum XLS Ultra is that it allows a much more selective isolation of a target pre-cursor ion at the first stage of MS (Q1). This allows further discrimination against intense matrix ions that appear at nominal precursor masses (isobaric interferences). As a general rule, in order to achieve this, precursor masses need to be specified more accurately to the instrument within 1 or 2 decimal places as well as the instrument mass resolution on Q1 being set to ≤ 0.2 Da FWHM. This is described as an "ultra-selective" mode for a quadrupole analyzer. Resolution settings can be increased for Q3 also for more selectivity in product ion experiments, although for SRM Q1, resolution is a more critical parameter.

Enhanced mass resolution with quadrupoles can be achieved by using precision machined hyperbolic quadrupoles of special length. Ultra-selective quadrupole mass resolution for SRM detection (U-SRM) is a novel acquisition mode for GC-MS/MS instruments. This mode allows the combination of increased mass resolution selectivity and structural selectivity when targeting compounds in complex matrix. The TSQ Quantum XLS Ultra allows this mode by incorporating extra long hyperbolic quadrupole rods (190 mm), with a wide internal radius of 6 mm, as shown in Figure 2. While the long rods deliver excellent mass peak form and resolution, the wide 6 mm radius accepts an increased number of ions from the source for increased ion transmission and sensitivity in the ultra selective SRM mode (U-SRM).

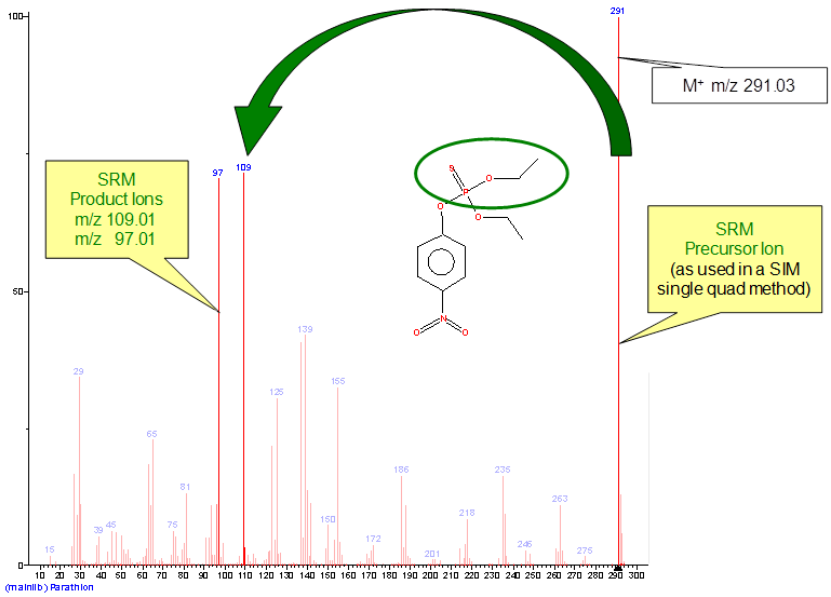

Figure. 4: EI mass spectrum of parathion simulating SRM precursor and product ions for MS/MS (NIST library)

Exact Mass and Mass Defect

Common target compounds for pesticides, drugs, or persistent organic pollutants (POPs) analyses typically contain a high number of heteroatoms or halogens in their structure. Typically hydrocarbon based or bio-organic compounds are forming common background matrix compounds. Examples of these include fuel oils, triglycerides, humic/fulvic substances, waxes, lignin structures or similar compound classes. In order to understand why ultra-selective precursor isolation increases selectivity in real applications when using U-SRM, it is necessary to visit the concept of exact mass and mass defect.

Exact mass is simply the calculation of the mass of a compound to a greater degree of accuracy. This is typically identifiable when masses are shown carry multiple decimal places. When this is measured value on a mass spectrometer, we refer to this as the accurate mass within a specified tolerance.

A closer look at the elemental composition of common target compounds detected in trace residue analysis reveals that, relative to carbon (with its IUPAC defined atomic weight of exact 12.00 g/mol), only hydrogen and nitrogen show a significant positive shift of its exact mass from the nominal mass of 1 g/mol and 14 g/mol respectively (see Table 1). Because of the high hydrogen occurrence in organic molecules, the apex of MS-detected mass peaks of hydrocarbons shift significantly on the accurate mass scale to higher masses. The calculated "mass defect" (in this case positive), is commonly expressed as a percentage of the deviation of the exact mass from its nominal value normalized to 100 Da, is typically in the range of 100 mDa/100Da for hydrocarbons.

In contrast to hydrogen, most heteroatoms, predominantly halogens, sulfur, phosphorous and silicone shift the mass peak of compounds containing these elements to lower masses. This can be described as a "negative mass defect." This fine difference in exact mass is used by the TSQ Quantum XLS Ultra to select target analytes during ultra selective acquisitions whilst discriminating against coeluting isobaric matrix ions. An example to illustrate this effect can be made for the pesticide HCB at the nominal mass *m/z* 282, see Table 2. The HCB mass peak is separated more than 0.5 Da on the mass scale from a nominally isobaric hydrocarbon background compound. This mass difference can be exploited to cleanly separate the HCB precursor ion in Q1 from the hydrocarbon matrix on the TSQ Quantum XLS Ultra. This is a relatively extreme example with a large delta mass. Depending on analyte/matrix combinations encountered, the resolving power of the quadrupole may need to exceed 5000+ resolution (FWHM). This is not usually available on standard triple quadrupole instruments that do not benefit from HyperQuad technology (see Figure 8).

Table 1: Mass defect of major elements in common analytes

Element	Nominal M [Da]	Exact M [Da]	Delta abs [Da]	Rel. Mass Defect [mDa/100Da]
C	12	12	0	0
H	1	1.0078	0.0078	783
N	14	14.0031	0.0031	22
O	16	15.9949	-0.0051	-32
O	16	15.9949	-0.0051	-32
S	32	31.9721	-0.0279	-87
Si	28	27.9769	-0.0231	-82
F	19	18.9984	-0.0016	-8
Cl	35	34.9689	-0.0311	-89
Br	79	78.9183	-0.0817	-103
I	127	126.9045	-0.0955	-75

Table 2: Example of the impact of the mass defect on the accurate mass at nominal mass *m/z* 282

Compound	Nominal M [Da]	Exact M [Da]	Delta abs [Da]	Rel. Mass Defect [mDa/100Da]
HCB				
C_6Cl_6	282	281.8134	-0.1866	-66
Alkane				
$C_{20}H_{42}$	282	282.3276	0.3276	116
Difference on mass scale 0.5142				

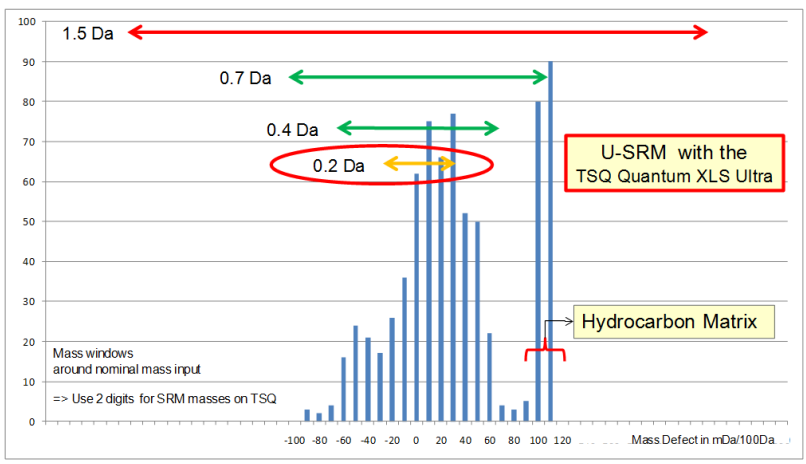

Figure 5: 700 Pesticides sorted according to frequency of their relative mass defect

This concept of enhanced mass resolution can be best visualized looking at the broad spectrum of pesticides with their inherent diversity of chemical compound classes. Figure 5 shows more than 700 pesticides and POPs compounds and their frequency distribution according to their "mass defect" values, see also the Thermo Fisher Scientific Pesticides Analyzer Reference Manual (Thermo Fisher Scientific, 2010). All of the compounds show a significant shift away from the organic hydrocarbon background to lower accurate masses due to their accurate mass.

The graphics in Figure 5 also shows the effect of increased mass resolution. The "selectivity window" with increasingly narrower mass peaks allows a good separation from hydrocarbon matrix interferences. Using a wider Q1 mass window beyond 0.7 Da with nominal or wide resolution setting of 1.5 Da will include the interfering matrix compounds into the fragmentation processes in the collision cell exhibiting high background noise. "Closing" the selectivity window to 0.2 Da using U-SRM on the TSQ Quantum XLS Ultra provides efficient selectivity for all shown compounds from matrix interferences.

Isolation of the Precursor Ion from Isobaric Matrix

Increased analyte selectivity is obtained during U-SRM with a narrow pre-selection of the precursor ion. The situation of having the pesticide lindane detected in a dirty matrix sample is shown in Figure 6 and Figure 7. During standard SRM operation, the Q1 nominal mass resolution of 0.7 Da (at the target mass *m/z* 219) transmits the lindane ion the collision cell for fragmentation along with a number of matrix ions. This can lead to isobaric interference and an increase in chemical noise. This problem can be exacerbated for systems that incorporated even wider precursor ion windows, such as the commonly seen "wide" or "open" resolution settings of > 1.0 Da FWHM. These wider settings can give an artificial impression of sensitivity, as the number of ions transmitted increases because noise can quickly appear in dirtier matrix samples.

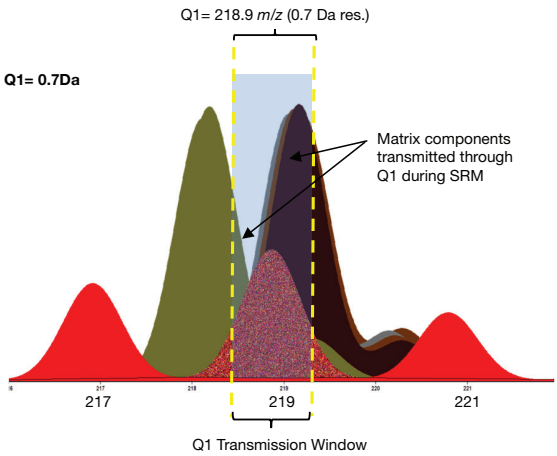

Figure 6: Precursor ion selection for lindane at 0.7Da FWHM (Q1) in standard SRM mode. Matrix components are transmitted to the collision cell during SRM acquisition.

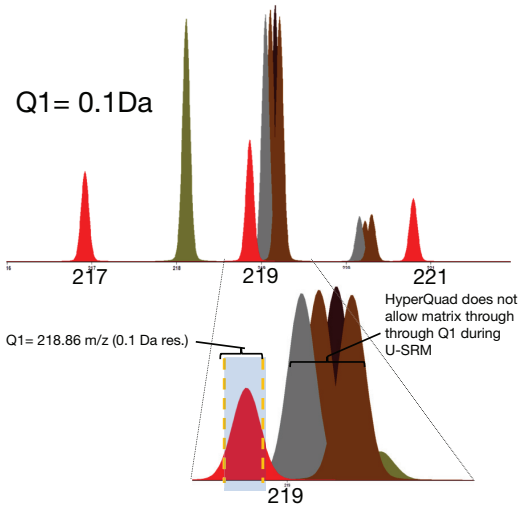

Figure 7: Precursor ion selection during U-SRM

In contrast to the standard or "wide" Q1 window setting, U-SRM "closes" the mass window in Q1 to < 0.2 Da, as seen in Figure 7. The increased resolution allows the lindane peak to be efficiently isolated for transmission to the collision cell in the absence of interfering matrix components. The resolution of the HyperQuad is such that any delta mass defect between target and matrix ions can be exploited. This is why U-SRM most efficiently eliminates isobaric interference effects on the precursor ion.

The difference in the mass resolution effect becomes even more evident comparing the resolution power of different triple quadrupole instrument types in Figure 8. The graph shows the calculated resolution over mass (peak width at half peak height, FWHM) for different mass peak width settings. Starting on the bottom from the "wide" setting with 1.2 Da peak width and the "standard" setting with

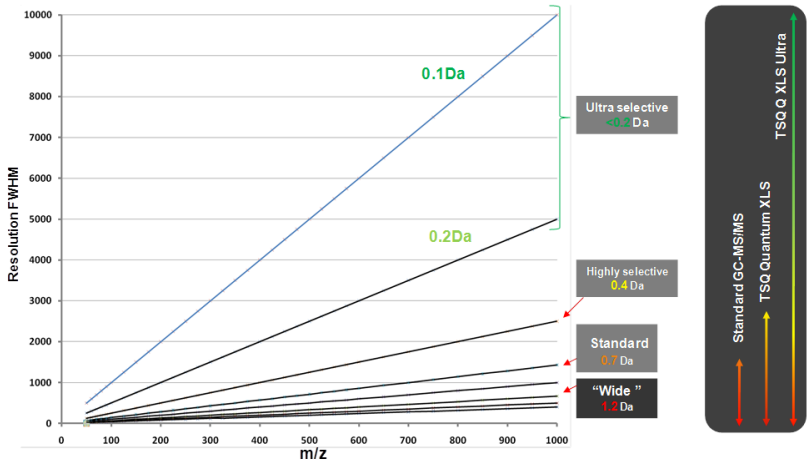

Figure 8: Mass resolution power of different instrument types

0.7 Da, there is already a remarkable resolution increase by using the highly selective setting with 0.4 Da peak with of the Thermo Scientific TSQ Quantum XLS. A significant increase in mass resolution is observed when progressing to the ultra selective mode with resolution settings of ≤ 0.2 Da when using the TSQ Quantum XLS Ultra.

Figure 9 extends the earlier example from Figure 3, octafluornapthalene in diesel, to compare the additional selectivity power of U-SRM. As already discussed, the use of increased resolution HyperQuads, operating in U-SRM mode, allows the possibility to further eliminate interference when moving to complex matrix. When compared to modes of operation that utilize "wide" or "open" quad resolution settings, it is clear that much higher confidence when addressing matrix samples can be taken with high sensitivity and high selectivity operation modes. In addition to this, it reminds us that we should endeavor to perform instrument evaluations in complex matrix samples with normalized instrument resolution settings. This allows both sensitivity and selectivity power to be observed.

Conclusions

Selectivity is a critical evaluation parameter for a GC-MS/MS system that is to face complex matrix samples. This is a key parameter for instrumental evaluation criteria alongside raw sensitivity and low-level precision performance.

GC-MS/MS using enhanced mass resolution mitigates the effect of surviving background interferences in SRM experiments, especially in complex sample matrices. High sensitivity, high selectivity analysis becomes possible, even with reduced clean-up procedures or direct Thermo Scientific Dionex ASE extracts for a large number of target compounds in one run.

The analytical advantages of using U-SRM on the TSQ Quantum XLS Ultra translate into increased productivity for routine analysis by the increasing data quality and increasing the possibility to save time with more generic sample preparation approaches. Reliable automatic peak integration becomes a regular feature of data analysis which allows a much reduced manual invention and faster time to result. This capability is particularly critical for laboratories with high sample throughput.

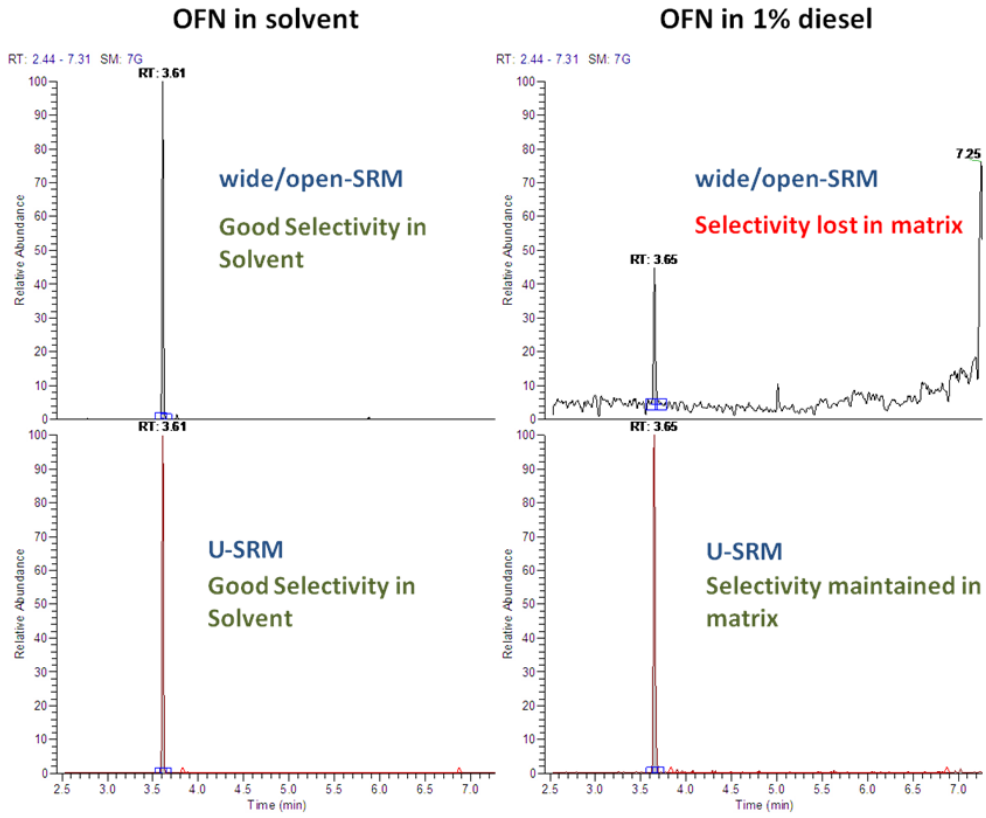

Figure 9. 100 fg/µL OFN in solvent (left) and 1% diesel (right) under "wide/open" SRM conditions (top) and ultra selective SRM mode (bottom)

Abbreviations

Da	International SI mass unit Dalton
CID	Collision-Induced Fragmentation (typically in the collision cell Q2)
FWHM	Full width at half maximum (a measure of the peak width at half peak height)
H-SRM	Highly selective SRM process (typically at 0.4 Da peak width)
HxCDD	Hexachlorodibenzodioxin
MRM	Multiple Reaction Monitoring (typically describing the analysis for multiple compounds)
OCP	Organochlorine pesticides
PCB	Polychlorinated biphenyl
PCDD/F	Polychlorinated dibenzodioxins and dibenzofurans
QuEChERS	Dispersive SPE extraction method, acronym for Quick Easy Cheap Efficient Rugged and Safe
SIM	Selected Ion Monitoring
SRM	Selected Reaction Monitoring (typically describing the technical process)
TSQ	Triple Stage Quadrupole instrument
U-SRM	Ultra selective SRM process (typically at peak widths at or below 0.2 Da peak widths)

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